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

A. 510(k) Number:

k081083

B. Purpose for Submission:

New assay

C. Measurand:

Mycophenolic Acid

D. Type of Test:

Quantitative homogeneous immunoassay

E. Applicant:

Thermo Fisher Scientific

F. Proprietary and Established Names:

CEDIA Mycophenolic Acid Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OAV	Sirolimus Test System (classification name)	862.3840	Toxicology
DLJ	Clinical Toxicology Calibrator	862.3200	Toxicology
LAS	Clinical Toxicology Control Material	862.3280	Toxicology

H. Intended Use:

1. Intended use(s):

    The CEDIA Mycophenolic Acid Assay is an *in vitro* diagnostic immunoassay intended for the quantitative determination of mycophenolic acid in human plasma or serum using automated clinical chemistry analyzers, as an aid in the management of mycophenolic acid therapy in renal and cardiac transplant patients.

    The CEDIA Mycophenolic Acid Calibrators are intended for use in the calibration of the CEDIA MPA Assay.

    The MAS Mycophenolic Acid Controls are intended for use as assayed quality control material for validation of MPA Assays.

2. Indication(s) for use:

    See intended use

3. Special conditions for use statement(s):

    The assay cross-reacts with AcMPAG, the acyl glucuronide metabolite of MPA (see cross-reactivity section, below) and may have an overall positive bias relative to reference methods or other assays that do not cross-react with metabolites. The relative bias in any particular patient sample depends in part on the metabolite concentration in that sample.

    Laboratories should include identification of the assay used on patient reports to aid in interpretation of results. Also see the Expected Values section, below.

    For prescription use only.

4. Special instrument requirements:

    Performance characteristics represented in the 510(k) were determined on the Hitachi 917.

I. Device Description:

The device consists of a set of reagents including anti-MPA polyclonal antibodies in buffer with preservatives and stabilizer, B-galactosidase "acceptor fragment" in buffer with preservatives, MPA conjugated B-galactosidase "donor fragment", chlorophenol red, and B-D-galactopyranoside with stabilizers and preservatives.

Calibrators

The CEDIA MPA Calibrator kit is comprised of liquid-ready to use, low and high calibrators, prepared in protein-based matrix containing stabilizers and less than 0.1% sodium azide as a preservative. The low calibrator contains no analyte; the high calibrator contains approximately 10 µg/mL MPA.

Controls
The MAS MPA Controls are liquid for ready-to-use controls prepared in plasma-based matrix consisting of human plasma, stabilizers and less than 0.1% sodium azide as a preservative. The 3 target concentrations for controls are 1.0, 2.5, and 6.0 µg/mL.

Materials of human origin, used in formulation of the MAS MPA controls, are tested for HIV1, HIV2, Hepatitis B, and Hepatitis C by FDA approved methods and confirmed as negative. However, as no test method can rule out infectious material with absolute certainty, the material must be handled as though infectious.

J. Substantial Equivalence Information:

1. Predicate device name(s): Roche Total Mycophenolic Acid Assay

2. Predicate K number(s): k063520

3. Comparison with predicate:
Both assays have the same intended use. The tests differ in terms of test principle: The CEDIA Mycophenolic Acid Assay is an immunoassay which utilizes antibodies to mycophenolic acid. The predicate device is based on enzyme-(inosine monophosphate dehydrogenase) inhibition and enzyme mimicking principles. The assay range for this device extends to 10 ug/mL. The range for the predicate device extends to 15 ug/mL.

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

EP5-A2, CLSI Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition, EP5-A2 (2004).

L. Test Principle:

The assay is based on the enzyme β-galactosidase, which has been genetically engineered into two inactive fragments termed enzyme donor (ED) and enzyme acceptor (EA). These fragments spontaneously re-associate to form fully active enzymes that, in assay format, cleave a substrate, generating a color change that can be measured spectrophotometrically. In the assay, analyte in the specimen competes with analyte conjugated to ED of β-galactosidase for limited numbers of antibody binding sites. If analyte is present in the sample, it binds to the antibody, leaving the ED conjugate free to form active enzymes with the EA. If analyte is not present in the sample, the antibody binds to analyte conjugated to ED, inhibiting the re-association of ED to EA, and no active enzyme is formed. The amount of active enzyme formed and resultant absorbance

change are directly proportional to the amount of drug present in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

    a. *Precision/Reproducibility:*

Precision was evaluated using pooled patient samples, spiked samples, and MAS MPA Controls. The two spiked samples (#1 and #3, in the table below) were prepared by adding MPA stock solution to negative plasma specimens. The pooled patient sample (#2, below) was prepared by combining specimens from transplant patients receiving mycophenolic acid therapy. The three controls were manufactured in a plasma-based matrix and spiked with MPA stock solution. Results were evaluated according to the CLSI EP5-A2 precision protocol. For each of the 21 runs, three cups of each sample were tested in duplicate to generate 6 data points for each sample per run. Calibration was performed for each run. Results are summarized in the following table.

	Sample 1	Sample 2	Sample 3	Control 1	Control 2	Control 3
N	126	126	126	126	126	126
Mean (µg/mL)	1.02	2.39	5.97	1.08	2.67	5.86
Within-run SD (µg/mL)	0.06	0.07	0.09	0.06	0.06	0.12
Within-run %CV	5.6	2.8	1.5	5.5	2.2	2.0
Total-run SD (µg/mL)	0.08	0.09	0.14	0.10	0.13	0.20
Total-run %CV	7.7	4.0	2.3	9.5	4.8	3.3

Similar precision was observed during external site studies, as shown in the table below. At external "site 1", six replicates were tested once a day for 5 days within a ten-day span (total n=30). At external "site 2", six replicates were tested twice a day for 5 days (total n=60).

	External site 1			External site 2		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
N	30	30	30	60	60	60
Mean (µg/mL)	1.02	2.62	6.06	0.99	2.49	5.7
Within-run SD (µg/mL)	0.07	0.06	0.10	0.08	0.09	0.11
Within-run %CV	6.6	2.3	1.7	7.9	3.6	2.0
Total-run SD (µg/mL)	0.08	0.07	0.15	0.08	0.09	0.15
Total-run %CV	7.7	2.9	2.5	8.4	3.8	2.7

b. Linearity/assay reportable range:

Results of the limit of quantitation and the linearity evaluations support the reportable range 0.3–10.0 ug/mL for this assay. This section describes linearity and spike/recovery studies. (Recovery and precision at the lower limit of the assay range are summarized in the Detection Limit section, below).

Linearity:
Linearity was evaluated by diluting "high pools" prepared from heart transplant patients and kidney transplant patients treated with MPA. The high pool was prepared to contain approximately 10 µg/mL MPA and was serially diluted with an MPA-negative sample to 8 concentration levels distributed evenly across the assay range. Samples were measured with the CEDIA assay in replicates (n=5). Measured concentrations were evaluated against expected concentrations. Expected concentrations were calculated from the measured value of the high sample and the dilution factor. The serially diluted samples recovered within 10% or 0.1 µg/mL. Results are tabulated below.

Heart Transplant Plasma Sample

Sample	Expected (µg/mL)	Measured (µg/mL))	% Recovery (Measured/Expected x 100%)	Difference (µg/mL) (Measured-Expected)
Level 1	--	9.8	-	-
Level 2	7.4	7.4	100.0	0.0
Level 3	4.9	4.9	100.0	0.0
Level 4	3.4	3.3	97.1	-0.1
Level 5	2.5	2.3	92.0	-0.2
Level 6	1.0	0.9	90.0	-0.1
Level 7	0.5	0.4	80.0	-0.1
Level 8	0.0	0.0	-	-
Regression equation: y=1.01x+0.08				

Kidney Transplant Plasma Sample

Sample	Expected (µg/mL)	Measured (µg/mL)	% Recovery (Measured/Expected x 100%)	Difference, (µg/mL) (Measured-Expected)
Level 1	10.2	10.2	-	-
Level 2	7.7	7.7	100.0	0.0
Level 3	5.1	5.0	98.0	-0.1
Level 4	3.6	3.4	94.4	-0.2
Level 5	2.6	2.4	92.3	-0.2
Level 6	1.0	0.9	90.0	-0.1
Level 7	0.5	0.5	100.0	0.0
Level 8	0.0	0.0	-	-

Regression equation: Y=1.01x-0.09

The manufacturer recommends that samples with MPA concentrations greater than 10 ug/mL (the upper limit of the assay) may be diluted 1:1 with 0 calibrator and retested. To evaluate recovery with this procedure eight patient samples with concentrations above 10 (µg/mL) were tested by diluting 1:1 (sample to diluent) according to the manufacturers recommendations in the package insert. Recovery results in this evaluation ranged from 103-111%, relative to expected concentrations (based on reference method results).

Spike Recovery:
To evaluate accuracy by recovery, a gravimetrically prepared MPA stock solution was spiked into both negative samples and patient samples already containing some MPA. Expected concentrations were calculated as the original MPA concentration (measured by CEDIA) plus the spiked MPA concentration (measured gravimetrically, independent of the CEDIA assay). Recoveries were within 10%, or 0.1 ug/mL. Results are summarized in the following tables.

MPA Negative Plasma Sample

Expected (µg/mL))	Measured (µg/mL)	% Recovery (Measured/Expected x 100%)
0.0	0.0	-
0.5	0.5	100.0
1.0	0.9	90.0
2.5	2.5	100.0
3.5	3.2	91.4
7.0	6.5	92.9

Heart Transplant Plasma Sample

Sample	Expected (µg/mL))	Measured (µg/mL)	% Recovery (Measured/Expected X 100%)	Difference (µg/mL) (Measured minus Expected conc.)
Sample 1	0.5	0.5	-	-
Sample 1 + 0.5	1.0	1.0	100.0	0.0
Sample + 2.0	2.5	2.6	104.0	0.1
Sample 2	2.4	2.4	-	-
Sample 2 + 1.0	3.4	3.3	97.1	-0.1
Sample 2 + 4.5	6.9	6.8	98.6	-0.1

Kidney Transplant Plasma Sample

Sample	Expected (μg/mL)	Measured (μg/mL)	% Recovery (Measured/Expected X 100%)	Difference (μg/mL) (Measured minus Expected conc.)
Sample 1	0.8	0.8	-	-
Sample 1 + 0.5	1.3	1.2	92.3	-0.1
Sample + 2.0	2.8	2.6	92.9	-0.2
Sample 2	2.3	2.3	-	-
Sample 2 + 1.0	3.3	3.2	97.0	-0.1
Sample 2 + 4.5	6.8	6.3	92.6	-0.5

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

Calibrators:
Calibrators are provided at 0 and 10 ug/mL. They are manufactured in a protein-based matrix of human plasma. The manufacturer's primary standards are traceable to commercially available MPA material prepared gravimetrically using MPA of greater than 99.5% purity. (No USP material is available). To assign values, lot calibrators are tested with the CEDIA MPA Assay across multiple instruments, using primary standards as calibrators. The manufacturer estimates the uncertainty in value assignment of lot calibrators relative to primary standards as 0.0 for the 0-level and 0.13ug/mL for the 10 ug/mL level.

Controls:
The 3-level set of controls (1.0, 2.5, and 6.0 ug/mL) are manufactured in plasma-based matrix and spiked using a characterized MPA stock solution. Control ranges are assigned using multiple lots, instruments and operators. The labeling clarifies that the ranges are provided only as a guide and that laboratories should establish their own acceptable ranges.

Stability:
Expiration dating was established by real time stability studies for both unopened and re-consituted calibrators. For each time point (n=10) , test calibrator was measured in replicates, using fresh, reconstituted reagent, and primary standards that had been stored at -80 degrees C. During testing, unopened calibrators were stored at 2-8 degrees C. No significant change in calibrator recovery was seen throughout the expiration dating period.

Opened vial testing was performed with fresh reconstituted calibrators stored at 2-8 degrees for the testing duration. Calibrator recovery at the end of expiration period was > 99%.

Stability studies are ongoing.

d. Detection limit:

Precision and accuracy of the assay near the low limits were evaluated. The study was carried out in 6 runs with 7 replicates per run over 28 days on 3 instruments for a total of 42 data points. Specimens from organ transplant patients receiving mycophenolic acid therapy were used. Individual patient plasma samples were combined to create pools with a gradient of MPA concentrations, values confirmed by LC-MS/MS. The samples were then dispensed as aliquots, stored at –20°C, and a single aliquot was thawed for each immunoassay run. Inter-assay imprecision observed at the lower limit of the assay (0.3 µg/mL) is < 18%, and calculated bias is < 0.1 at this concentration. Results are summarized in the following table.

Expected Conc. (ug/mL)	0.13	0.25	0.50	1.00	2.00	4.00
CEDIA n =	42	42	42	42	42	42
Mean (µg/mL)	0.12	0.26	0.56	1.10	2.13	4.22
Bias (ug/mL)	0.01	0.01	0.06	0.1 (110%)	0.13 (107%)	0.22 (106%)
SD (µg/mL)	0.05	0.05	0.07	0.07	0.11	0.10
%CV	**43.0**	**17.6**	**12.5**	**6.7**	**5.2**	**2.3**

e. Analytical specificity:

Metabolite cross-reactivity:
The cross-reactivity of the CEDIA MPA Assay with 7-O-Glucuronide MPA (MPAG) and Acyl Glucuronide MPA (AcMPAG) was evaluated. MPA metabolites were added into both MPA-negative plasma and plasma containing various concentrations of MPA (see below). Concentrations of metabolite were determined from gravimetric measurement. Purities were determined by HPLC and Mass Spectral analysis. The spiked samples were tested by the CEDIA MPA Assay in duplicate and cross reactivity was calculated using the formula: % Cross reactivity = (Measured [MPA] - Expected [MPA]) / Spiked [Metabolite] x 100%

AcMPAG cross-reactivity observed from this evaluation ranged from 144 to 178 %. See table below. No cross reactivity (< 0.1%) was found to MPAG at concentrations as high as 50-1000 ug/mL.

Metabolites

Conc of AcMPAG in the sample	Conc. Of MPA (ug/mL)	Measured MPA (ug/mL)	Difference between measured and expected MPA (ug/mL)	Percent cross-reactivity
10.0	3.0	19.4	16.4	164.4
1.8	7.8	10.4	2.6	144.4
0.9	3.2	4.8	1.6	177.8
0.3	1.4	1.8	0.4	133.3
3.0	0.0	5.1	5.1	170.0

Cross Reactivity with Other Immunosuppressants:
Sirolimus, tacrolimus, and cyclosporine were tested for cross reactivity with the CEDIA MPA Assay. Samples were prepared by adding the other immunosuppressants at concentrations ranging up to 300 ng/mL (sirolimus and tacrolimus) and 1000 ng/mL (cyclosporine) into plasma containing MPA. Corresponding controls were prepared by adding the same volume of solvent that was in the stock solution of the compound. Samples were assayed in triplicate for MPA. No significant interference was observed. (Differences in MPA recovery between test and control samples were ≤ 0.1 ug/mL)

Cross Reactivity with Common Drugs:
Drugs that may be commonly used with mycophenolic acid were tested for cross reactivity with the CEDIA MPA Assay. Samples were prepared, by adding high concentrations of the drugs to MPA-negative plasma, as well as plasma containing 2.2 ug/mL MPA, and then tested in duplicate by CEDIA. Corresponding controls were prepared by adding the same volume of solvent that was in the stock solution of the compound. Bias between control samples and test samples were generally < 0.1 ug/mL, indicating no interference under these conditions. A small bias (0.25 ug/mL) was observed with high concentrations (50 ug/mL) of amphotericin B.   A full list of the drugs tested is included in the package insert.

Interference by Endogenous Substances:
Potential interference effects by high levels of bilirubin (20 mg/dL), triglycerides (1600 mg/dL), uric Acid (233 mg/dL), and hemoglobin (1 g/dL) were evaluated. Compounds were added directly to MPA-negative plasma at target concentrations, as well as to samples containing MPA at concentratiosn of 1.0, 2.5, and 6.0 ug/mL MPA. Naturally occurring samples high in rheumatoid factor were also tested. Controls were prepared by adding the same volume of solvent that was used to make the stock solution of the compound being tested. Sample recoveries in this evaluation were all within +/- 10% and no trends were observed due to endogenous compounds.
Samples containing high concentrations of cholesterol (up to 400 mg/dL) and total protein (up to 11 mg/dL) from transplant patients receiving mycophenolic acid therapy were also evaluated. Recoveries relative to expected values were within +/-10%.

  f. *Assay cut-off:*

     Not applicable; this is a quantitative assay.

 2. Comparison studies:

  a. *Method comparison with predicate device:*

Leftover and unidentifiable clinical K2-EDTA plasma samples from heart and kidney transplant patients receiving either mycophenolate mofetil or mycophenolate sodium therapy were obtained from a clinical site. The samples were tested at external sites and the manufacturer's site with the CEDIA assay and with a reference method. (Information about the reference methods was included in the 510(k)). Samples were pre-dose

samples from adult patients. Co-administered drugs included tacrolimus (n= 153), and cyclosporine (n=34). A more positive bias was observed for the latter samples in this study, and this is illustrated in the graph in the package insert. Results of the regression analyses based on data obtained at the manufacturer's site, as well as an external site, are shown below:

Analysis of data from the manufacturer's site:

Transplant Type	Regression Type	Slope (95% CI range)	Intercept (95% CI Range)	Correlation (r-value)	N
Heart	Least	1.114 (1.061 to 1.166)	0.20 (0.05 to 0.36)	0.9743	96
	Deming	1.147 (1.094 to 1.200)	0.12 (-0.04 to 0.28)		
Kidney	Least	1.027 (0.974 to 1.080)	0.16 (-0.03 to 0.36)	0.9711	92
	Deming	1.060 (1.006 to 1.113)	0.06 (-0.13 to 0.25)		
Heart and Kidney	Least	1.054 (1.015 to 1.092)	0.22 (0.09 to 0.34)	0.9698	188
	Deming	1.089 (1.051 to 1.128)	0.12 (-0.01 to 0.25)		

Analysis of data from an external site:

Transplant	Regression	Slope (95% CI Range)	Intercept (95% CI Range)	Correlation	N
Heart	Least	0.993 (0.924 to 1.062)	0.39 (0.19 to 0.60)	0.9472	96
	Deming	1.051 (0.982 to 1.121)	0.25 (0.04 to 0.47)		
Kidney	Least	0.973 (0.921 to 1.025)	0.14 (-0.05 to 0.33)	0.9689	92
	Deming	1.005 (0.952 to 1.057)	0.04 (-0.15 to 0.23)		
Heart and Kidney	Least	0.966 (0.922 to 1.010)	0.32 (0.17 to 0.46)	0.9540	188
	Deming	1.013 (0.968 to 1.057)	0.18 (0.03 to 0.33)		

Method comparison of CEDIA assay at external site versus manufacturer's site:

Transplant Type	Regression Type	Slope (95% CI Range)	Intercept (95% CI Range)	Correlation (r-value)	N
Heart and Kidney	Least Square	0.976 (0.968 to 0.985)	-0.07 (-0.10 to -0.04)	0.9982	188
	Deming	0.978 (0.970 to 0.987)	-0.08 (-0.11 to -0.05)		

Bias plots included in the package insert illustrate the comparison between the reference method and CEDIA for this patient population. The mean bias (y-x) = 0.37 ug/mL; SD = 0.47 ug/mL. (Mean bias +1.96 SD = 1.29 ug/mL; Mean bias -1.96 SD = -0.55 ug/mL).

b. Matrix comparison:

A split sample matrix comparison study was conducted to compare K_2EDTA samples, used in the method comparison study, to Na_2EDTA, Na Heparin, Li Heparin, and NH_4 Heparin. Twenty-one samples across the assay range were prepared by spiking MPA into each of paired matrix samples. The results of samples prepared in the evaluating matrix were compared to those in K_2EDTA plasma by determining the slope, intercept and correlation coefficient, as well as recoveries relative to K_2EDTA samples.

Method Comparison	Method	N	Conc. Range	Slope (Deming's)	Intercept	R
1	X: K_2EDTA Y: Na_2EDTA	21	0.6-9.6	0.983	-0.02	0.9947
3	X: K_2EDTA Y: Na Heparin	21	0.5-9.7	0.981	0.07	0.9982
4	X: K_2EDTA Y: Li Heparin	21	0.5-9.9	1.017	0.00	0.9971
5	X: K_2EDTA Y: NH_4 Heparin	21	0.4-8.2	0.992	0.00	0.9977

Recoveries at each concentration were within +/- 10%, or +/-0.2 ug/dL.

3. Clinical studies:

Clinical studies are not typically called for with this type of assay.

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:

See Expected Values, below.

5. Expected values/Reference range: The following is included in the package insert:

The optimal therapeutic range for MPA in plasma has not been fully established. In addition, optimal patient MPA concentration ranges may vary depending on the specific assay and its metabolite cross-reactivities, (See cross-reactivity section, for observed cross-reactivities with this assay). Therefore, optimal ranges should be established for each commercial test and values obtained with different assay methods cannot be used interchangeably, nor should correction factors be applied. Laboratories should include identification of the assay used on patient reports in order to aid in interpretation of results.

Optimal ranges depend upon transplant type and co-administered drugs, as well as the patient's clinical state, individual differences in sensitivity to immunosuppressive and toxic effects of MPA, time post-transplant and a number of other factors. Individual MPA values cannot be used as the sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made. Each institution should establish the optimal ranges based on the specific assay used and other factors relevant to its patient population.

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 substantial equivalence decision.