



Memorandum

Date OCT - 2 1996

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of Behring Diagnostic's (formerly Syva Company)
Emit®2000 Cyclosporine Specific Assay

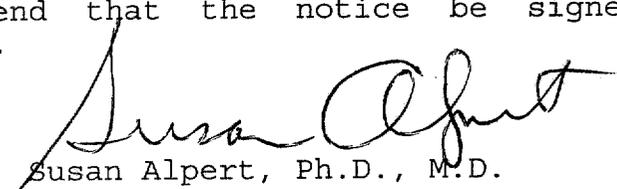
To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

- (1) a premarket approval order for the above referenced medical device (Tab B); and
- (2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.


Susan Alpert, Ph.D., M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved _____ Disapproved _____ Date _____

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

[DOCKET NO. _____]

Behring Diagnostics, Inc.; PREMARKET APPROVAL OF EMIT®2000 Cyclosporine Specific Assay

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Behring Diagnostics, Inc., San Jose, CA., for premarket approval, under the Federal Food, Drug, and Cosmetic Act (the act), of EMIT®2000 Cyclosporine Specific Assay. After reviewing the recommendation of the Clinical Chemistry and Toxicology Devices Panel, FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter on October 2, 1996, of the approval of the application.

DATES: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESSES: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review, to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857.

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FOR FURTHER INFORMATION CONTACT:

Cornelia Rooks,
Center for Devices and Radiological Health (HFZ-440),
Food and Drug Administration,
9200 Corporate Blvd .
Rockville, MD 20850,
301-594-3084.

SUPPLEMENTARY INFORMATION: On June 29, 1992, Syva Co., San Jose, CA. 95161-9013, submitted to CDRH an application for premarket approval of the EMIT®2000

Cyclosporine Specific Assay The device is a homogeneous enzyme immunoassay and is indicated for in vitro diagnostic use on the Roche Diagnostic Systems, COBAS MIRA®, COBAS MIRA S®, and COBAS MIRA® Plus chemistry systems for the quantitative analysis of cyclosporine (CsA) in human whole blood as an aid in the management of cyclosporine therapy in kidney, heart, and liver transplant patients.

On November 16, 1992, the Clinical Chemistry and Toxicology Devices Panel of the Medical Devices Advisory Committee, an FDA advisory committee, reviewed and recommended approval of the application.

On October 2, 1996, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

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A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

Opportunity For Administrative Review

Section 515(d)(3) of the act, (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act, for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and procedures regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

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Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h) (21 U.S.C. 360e(d), 360j(h))) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated: _____.

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Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

Mr. Paul Rogers, Jr.
Manager, Regulatory Affairs
Behring Diagnostics, Inc.
P.O. Box 49013
San Jose, California 95161-9013

OCT - 2 1996

Re: P920031
Emit[®] 2000 Cyclosporine Specific Assay
Filed: June 29, 1992
Amended: July 28, October 13, October 26, and December 23,
1992; February 5, February 12, February 27 and September 29,
1993; October 10, November 22, 1995; April 11,
June 17, June 27 and August 26, 1996.

Dear Mr. Rogers:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Emit[®] 2000 Cyclosporine Specific Assay. This device is indicated for in vitro diagnostic use on the Roche Diagnostics Systems COBAS MIRA, COBAS MIRA S, and COBAS MIRA Plus chemistry systems for the quantitative analysis of cyclosporine (CsA) in human whole blood as an aid in the management of cyclosporine therapy in kidney, heart, and liver transplant patients. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

Expiration dating for this device has been established and approved at 9 months when stored at 2° to 8° C.

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

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Page 2 -Mr. Paul Rogers, Jr.

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

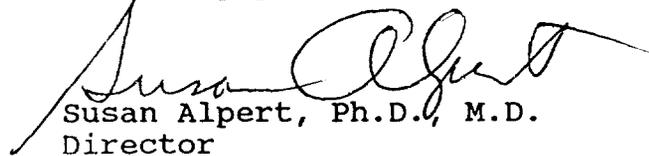
You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Cornelia Rooks at (301) 594-1243.

Sincerely yours,



Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

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CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effectuated" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the addition of, but not the replacement of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

- (1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).
- (2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:
 - (a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

- (b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

- (1) A mixup of the device or its labeling with another article.
- (2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and
 - (a) has not been addressed by the device's labeling or
 - (b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.
- (3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984, and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to FDA whenever they receive or otherwise become aware of information that reasonably suggests that one of its marketed devices

- (1) may have caused or contributed to a death or serious injury or
- (2) has malfunctioned and that the device or any other device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for this PMA, you shall submit the appropriate reports required by the MDR Regulation and identified with the PMA reference number to the following office:

Division of Surveillance Systems (HFZ-531)
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, Room 240
Rockville, Maryland 20850
Telephone (301) 594-2735

Events included in periodic reports to the PMA that have also been reported under the MDR Regulation must be so identified in the periodic report to the PMA to prevent duplicative entry into FDA information systems.

Copies of the MDR Regulation and an FDA publication entitled, "An Overview of the Medical Device Reporting Regulation," are available by written request to the address below or by telephoning 1-800-638-2041.

Division of Small Manufacturers Assistance (HFZ-220)
Center for Devices and Radiological Health
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

I General Information

Generic Name: *In vitro* reagent system for the quantitative measurement of cyclosporine in human whole blood samples.

Trade Name: Emit® 2000 Cyclosporine Specific Assay

Applicant's Name and Address:

Behring Diagnostics Inc.
3403 Yerba Buena Road
P.O. Box 49013
San Jose, CA 95161-9013

Premarket Approval Application (PMA) Number: P920031

Date of Panel Recommendation: November 16, 1992

Date of Notice of Approval to the Applicant: OCT - 2 1996

II. Indications for Use

The Emit® 2000 Cyclosporine Specific Assay is for *in vitro* diagnostic use on the Roche Diagnostics Systems COBAS MIRA, COBAS MIRA S, and COBAS MIRA Plus chemistry systems for the quantitative analysis of cyclosporine (CsA) in human whole blood as an aid in the management of cyclosporine therapy in kidney, heart, and liver transplant patients.

Contraindications: There are no known contraindications for the Emit® 2000 Cyclosporine Specific Assay.

Warnings: No firmly established therapeutic range exists for effective cyclosporine concentration in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of cyclosporine, coadministration of other immunosuppressants, type of transplant, time post transplant, and a number of other factors will result in different requirements for optimal blood levels of cyclosporine. Individual cyclosporine values can not be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made.

Clinical response to cyclosporine treatment does not correlate well with the administered dose. Absorption and clearance of cyclosporine can vary greatly among patients. Factors affecting cyclosporine concentrations in blood include the nature of the transplant, the age of the patient, the general health of the patient (specifically the presence or absence of liver disease or

gastrointestinal disfunction), the patient's lipoprotein profile and hematocrit, and the coadministration of cyclosporine with food or with certain drugs (1, 2).

Several drugs may lower cyclosporine concentrations through induction of cytochrome P-450 enzymes. These include phenytoin, phenobarbital, carbamazepine, and rifampin. Other drugs may increase through cyclosporine levels (e.g. ketoconazole, high-dose methylprednisolone, erythromycin, and deltiagem). Monitoring cyclosporine levels becomes even more important when concurrent drugs are initiated or withdrawn (3).

Precautions: Precautions for use of the device are stated in the attached product labeling (Attachment A).

Background

Cyclosporine (CsA) is a cyclic undecapeptide of fungal origin and a potent immunosuppressive agent. Since its introduction in 1983, cyclosporine has improved the patient and graft survival in patients receiving heart, kidney, or liver transplants. Studies have documented the effect of cyclosporine in combating organ rejection by reducing the frequency of complications (4).

A continuing trend exists towards the use of whole blood, instead of plasma, as the matrix of choice for the measurement of cyclosporine (5). The National Academy of Clinical Biochemistry/American Association for Clinical Chemistry Task Force on Cyclosporine Monitoring specifically recommended using whole blood in its 1987 report (1). The Task Force on Cyclosporine Monitoring also recommended that the method for measurement of cyclosporine be specific. The contributions of the more than 30 cyclosporine metabolites to immunosuppression or toxicity remains uncertain (1,3)

Cyclosporine has a narrow range for safe and effective therapy. Inadequate cyclosporine doses and levels may result in rejection of the transplanted organ. Toxic levels of cyclosporine are associated with many serious side effects, including nephrotoxicity, hepatotoxicity, and a range of other complications. Concern exists with the nephrotoxic effects of the drug when used in renal transplantation because of the difficulty in distinguishing between organ rejection and cyclosporine toxicity (3,6).

Monitoring parent drug cyclosporine concentrations in whole blood and interpreting these concentrations in conjunction with other laboratory data and clinical considerations is the most effective means of ensuring adequate immunosuppressant therapy for recipients of solid-organ transplants (7).

III. Device Description

The Emit® 2000 Cyclosporine Specific Assay employs a homogeneous enzyme immunoassay technique used for the analysis of cyclosporine in whole blood. The assay contains a mouse

monoclonal antibody with specificity to cyclosporine and a second mouse monoclonal antibody specific for a major metabolite of cyclosporine, AM9 (M1). The purpose of this second antibody is to bind to the metabolite and prevent it from binding to the cyclosporine antibody (8).

The Emit® 2000 Cyclosporine Specific Assay is based on competition for cyclosporine antibody binding sites. Cyclosporine in the sample competes with cyclosporine in Enzyme Reagent B that is labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH). Active (unbound) enzyme converts the oxidized nicotinamide adenine dinucleotide (NAD) in Antibody Reagent A to NADH, resulting in an absorbance change that can be measured spectrophotometrically. Enzyme activity decreases upon binding to the antibody, allowing the cyclosporine concentration in the sample to be measured in terms of enzyme activity. Endogenous serum G6P-DH does not interfere because the coenzyme NAD functions only with the bacterial (*Leuconostoc mesenteroides*) enzyme employed in the assay.

Before testing with the Emit® 2000 Cyclosporine Specific Assay, samples, calibrators, and controls are pretreated with methanol. Methanol lyses the cells, solubilizes the cyclosporine, and precipitates most of the blood proteins. The samples are centrifuged, and an aliquot of the resulting supernatant containing cyclosporine is diluted with Emit® 2000 Cyclosporine Specific Assay Diluent. This solution is then assayed using Reagents A and B on the COBAS MIRA or COBAS MIRA S or COBAS MIRA Plus chemistry system.

IV. Alternative Practices and Procedures

Alternative practices for the quantitative measurement of cyclosporine concentrations in blood include high performance liquid chromatography (HPLC), radioimmunoassay (RIA), and fluorescence polarization immunoassay (FPIA). HPLC and some polyclonal antibody-based immunoassay values are not interchangeable because the immunoassay methodologies measure cyclosporine and metabolites whereas HPLC measures cyclosporine only. Immunoassays specific to parent cyclosporine have been developed which correlate better to HPLC.

V. Marketing History

The Emit® 2000 Cyclosporine Specific Assay has been marketed in the following countries: Australia, Belgium, Canada, Cyprus, Denmark, France, Germany, Greece, India, Italy, Portugal, Netherlands, New Zealand, Saudi Arabia, South Africa, Switzerland, Spain, Sweden, and United Kingdom.

The Emit® 2000 Cyclosporine Specific Assay has not been withdrawn from the market or had corrective actions taken in any country for any reason related to the safety or effectiveness of this device.

VI. Adverse Effects of the Device on Health

A falsely elevated cyclosporine assay result could lead to a decreased dosage of cyclosporine and thereby increase the risk of transplant organ rejection. A falsely decreased cyclosporine assay result could lead to an increased cyclosporine dosage and thereby increase the risk of untoward side effects.

VII. Summary of Studies

A. Nonclinical Studies

Nonclinical laboratory studies for the evaluation of the Emit® 2000 Cyclosporine Specific Assay were conducted at Behring Diagnostics Inc.'s facilities in Palo Alto and San Jose, California. The Emit® 2000 Cyclosporine Specific Assay was performed on the Roche Diagnostics Systems COBAS MIRA, COBAS MIRA S, and COBAS MIRA Plus chemistry systems.

The reagent and diluent formulations used in the nonclinical studies were the same formulations used in the clinical studies. In the first set of nonclinical studies, three different lots of Calibrators were combined with six different lots of Reagents. In the second set of nonclinical studies, three other lots of Calibrators were paired with three other lots of Reagents. The Reagents and Calibrators lots used in the second set of clinical studies are collectively referred to as the "Three CsA Lots".

1. Precision

a. Total Precision (Stored Curve)

A standard curve was generated, validated with control quantitation, and then stored. Freshly prepared extracts of Level 1 (82.2 ng/mL) and Level 3 (367.3 ng/mL) controls were assayed as samples fifteen times, over a twenty-four day period and quantitated from the stored curve. The mean concentration (N = 16) and coefficient of variation (CV) values for each control were calculated.

The mean concentrations of the controls as well as CVs were comparable to the total precision calculated from concurrent curves. The CVs were 6.6 and 6.3 for Levels 1 and 3, demonstrating that a stored curve can be used to quantitate patient samples provided the curve is first validated.

b. Precision Within Run

Precision was evaluated in a manner consistent with the National Committee for Clinical Laboratory Standards (NCCLS) guideline, EP5-T2 (9), using tri-level controls. Three distinct extracts were prepared from each of the three control levels (Levels 1 to 3) for each run, and 2 runs per day for 20 days were performed. These data were collected on three analyzers (one

MIRA, one MIRA S and one MIRA Plus) with the Three CsA Lots.

Table 1 summarizes the Within-Run component of precision across all tested analyzers and the three Emit® 2000 Cyclosporine Specific Assay reagent and calibrator lots.

Table 1
Within-Run Precision for Three Emit® 2000 Cyclosporine Specific Assay Lots

MIRA Model	MIRA			MIRA S			MIRA Plus (#2)			
	Reagent Lot	Y2/Y3	Y3/Y4	Y4/Y5	Y2/Y3	Y3/Y4	Y4/Y5	Y2/Y3	Y3/Y4	Y4/Y5
Level 1										
Mean (ng/mL)		72.7	69.9	72.5	69.5	61.4	65.1	62.9	62.5	57.2
SD		4.56	3.55	4.01	5.18	4.04	4.38	4.54	3.73	3.52
CV (%)		6.3	5.1	5.5	7.5	6.6	6.7	7.2	6.0	6.2
N		120	120	120	120	120	120	120	120	120
Level 2										
Mean (ng/mL)		175.4	167.8	173.4	175.8	162.7	164.4	167.6	164.3	160.5
SD		6.40	4.98	4.79	7.60	5.75	6.25	6.89	5.54	6.27
CV (%)		3.6	3.0	2.8	4.3	3.5	3.8	4.1	3.4	3.9
N		120	120	120	120	120	120	120	120	120
Level 3										
Mean (ng/mL)		422.2	391.7	404.0	417.2	385.2	388.9	410.9	402.6	386.4
SD		14.54	11.28	12.48	19.03	11.64	11.89	16.67	18.95	12.34
CV (%)		3.4	2.9	3.1	4.6	3.0	3.1	4.1	4.7	3.2
N		120	120	120	120	120	120	120	120	120

The Within-Run Precision of the three CsA Lots were generally consistent between each other on all three MIRA models, and did not exceed a CV greater than 7.5 per cent in this study. They indicated that the Within-Run Precision obtained with any one of the models of the COBAS MIRA analyzer is acceptable

c. Total Precision

Table 2 summarizes Total Precision across all tested analyzers and the three Emit® 2000 Cyclosporine Specific Assay reagent and calibrator lots.

Table 2
Total Precision for Three Emit® 2000 Cyclosporine Specific Assay Lots

MIRA Model	MIRA			MIRA S			MIRA Plus (#2)		
	Y2/Y3	Y3/Y4	Y4/Y5	Y2/Y3	Y3/Y4	Y4/Y5	Y2/Y3	Y3/Y4	Y4/Y5
Level 1									
Mean (ng/mL)	72.7	69.9	72.5	69.5	61.4	65.1	62.9	62.5	57.2
SD	8.04	6.42	6.00	7.21	7.81	6.39	8.34	9.87	8.97
CV (%)	11.1	9.2	8.3	10.4	12.7	9.8	13.3	15.8	15.7
N	120	120	120	120	120	120	120	120	120
Level 2									
Mean (ng/mL)	175.4	167.8	173.4	175.8	162.7	164.4	167.6	164.3	160.5
SD	10.13	9.56	9.78	10.80	12.22	8.77	11.25	12.56	9.50
CV (%)	5.8	5.7	5.6	6.1	7.5	5.3	6.7	7.6	5.9
N	120	120	120	120	120	120	120	120	120
Level 3									
Mean (ng/mL)	422.2	391.7	404.0	417.2	385.2	388.9	410.9	402.6	386.4
SD	24.05	22.46	22.01	23.01	24.89	20.57	23.48	29.58	23.53
CV (%)	5.7	5.7	5.4	5.5	6.5	5.3	5.7	7.3	6.1
N	120	120	120	120	120	120	120	120	120

The Total Precision of the three CsA Lots were generally consistent between each other on all three MIRA models, and did not exceed a CV greater than 15.8 per cent in this study for Level 1, greater than 7.6 per cent for Level 2, and greater than 7.3 per cent for Level 3. The higher Total Precision CVs for Level 1, relative to Levels 2 and 3, reflect the higher Within-Run Precision CVs observed with Level 1 in the Within-Run Precision Study

The Total Precision obtained for Level 1 should not have any significant clinical impact as the lower limit of any commonly used therapeutic window, at any time post-transplant, is not likely to be less than 100 ng/mL of cyclosporine. The Total Precision obtained at Levels 2 and 3 (within therapeutic levels) was less than 8 per cent.

d. Within-Run Precision (Over Time)

Four sites evaluated within-run precision of the Emit® 2000 Cyclosporine Specific Assay by analyzing three different levels of controls with one lot of Emit reagents and calibrators (reagent lot D3 and calibrator lot D2). Each control level was analyzed 20 times on a separate run. At the end of the clinical trial, each site was again evaluated for within-run precision using the same reagents, calibrators, and controls, as a part of the between-lot evaluation. Again, each control level was analyzed 20 times in a separate calibrated run.

Table 3 summarizes the within-run precision results from the beginning and end of the trial at each site.

Table 3
Summary of Within-Run Precision Overtime
Results at the Beginning and End of the Trial for Four Sites

	Beginning of Trial	End of Trial	Beginning of Trial	End of Trial	Beginning of Trial	End of Trial
Trial Site	Control Level 1	Control Level 1	Control Level 2	Control Level 2	Control Level 3	Control Level 3
U. of Alabama						
Mean (ng/mL)	99.7	98.8	216.4	242.5	368.8	410.0
SD	6.1	7.5	14.8	8.1	17.4	28.6
CV (%)	6.1	7.6	6.9	3.3	4.7	7.0
U. of Cincinnati						
Mean (ng/mL)	86.2	96.2	221.5	218.3	398.9	386.1
SD	8.5	8.9	12.2	12.9	18.1	27.8
CV (%)	9.9	9.2	5.5	5.9	4.5	7.2
U. of Washington						
Mean (ng/mL)	95.4	89.5	221.2	219.7	402.5	408.0
SD	7.4	8.6	9.1	10.2	26.8	19.9
CV (%)	7.8	9.6	4.1	4.7	6.7	4.9
Mayo Clinic						
Mean (ng/mL)	89.5	92.1	216.2	231.8	402.3	433.8
SD	7.4	7.1	11.1	10.0	31.3	25.9
CV (%)	8.3	7.7	5.1	4.3	7.8	6.0

The ranges of the mean cyclosporine levels at the beginning of the trial, for the three Control Levels were: 86.2 to 99.7 ng/mL (Level 1), 216.2 to 221.5 ng/mL (Level 2), and 368.8 to 402.5 (Level 3). The assay showed good within-run precision with all CVs less than 10 per cent.

The ranges of the mean cyclosporine levels at the end of the trial, for the three Control Levels were: 89.5 to 98.8 ng/mL (Level 1), 218.3 to 242.5 ng/mL (Level 2), and 386.1 to 433.8 (Level 3). Precision CV changes over the course of a six-month study with all CVs observed at the end of the trial, again were less than 10 per cent.

e. Total Precision: Emit® 2000 Cyclosporine Specific Assay (4 Sites, 3 Lots)

A precision study was performed with the Emit® 2000 Cyclosporine Specific Assay, according to NCCLS. Three controls were assayed on every Emit run at four sites. In general two replicates of each control level were available from each valid run. Runs were declared to be invalid based on initially established control limits and adherence to Westgard rules.

Data from all valid runs were used in these analyses. For each control level, the mean, standard deviation (SD), and CV per cent of all data points were computed.

Table 4
First Total Precision Study Summary
for Four Clinical Trial Sites

Trial Site	Control Level 1	Control Level 2	Control Level 3
Mayo Clinic			
Mean (ng/mL)	92.5	222.8	390.1
SD (ng/mL)	10.36	20.59	30.63
CV (%)	11.2%	9.2%	7.9%
N	54	54	54
U. of Alabama			
Mean (ng/mL)	96.7	217.1	374.7
SD (ng/mL)	10.91	21.08	43.54
CV (%)	11.3%	9.7%	11.6%
N	79	80	79
U. of Cincinnati			
Mean (ng/mL)	93.8	212.7	381.3
SD (ng/mL)	10.65	18.90	39.57
CV (%)	11.4%	8.9%	10.4%
N	102	102	101
U. of Washington			
Mean (ng/mL)	95.0	222.9	397.2
SD (ng/mL)	8.91	14.45	28.83
CV (%)	9.4%	6.5%	7.3%
N	145	144	145

The mean cyclosporine concentrations at all four sites, for all Reagent lots, for the Level 1 Control, ranged from 92.5 ng/mL to 96.7 ng/mL, with CVs from 9.4 per cent to 11.4 per cent. For the Level 2 Control, the mean cyclosporine values ranged from 212.7 ng/mL to 222.9 ng/mL, with CVs from 6.5 per cent to 9.7 per cent. For the Level 3 Control, the mean cyclosporine values ranged from 374.7 ng/mL to 397.2 ng/mL with CVs from 7.3 per cent to 11.6 per cent. The summary statistics described above showed comparable results among the three combinations of Reagents/Calibrators lots, for each Control Level, and among the four sites.

f. Total Precision Emit® 2000 Assay (3 sites, 3 lots)

In the second total precision study, precision was evaluated in a manner consistent with the NCCLS guideline, EP5-T2, using tri-level controls. Three Reagents/Calibrators combination lots,

different from those involved in the first total precision study, were used at three study sites. Table 5 summarizes the second total precision results across three study sites and for the three CsA Lots.

Table 5
Second Total Precision Study Summary for Three Study Sites

Control Level	Univ. of Alberta Range Across 3 Lots	UCLA Range Across 3 Lots	Univ. of Washington Range Across 3 Lots
Level 1			
Mean (ng/mL)	71.6 to 76.2	63.3 to 73.0	71.4 to 77.8
SD	9.5 to 13.8	7.6 to 11.0	8.3 to 12.9
CV (%)	13.2% to 19.1%	10.4% to 17.3%	11.7% to 16.6%
Level 2			
Mean (ng/mL)	179.5 to 181.3	166.7 to 179.9	177.2 to 187.4
SD	15.6 to 16.7	11.5 to 16.8	10.7 to 15.6
CV (%)	8.6% to 9.3%	6.4% to 10.0%	6.0% to 8.8%
Level 3			
Mean (ng/mL)	402.6 to 427.2	390.6 to 429.8	409.1 to 436.1
SD	26.9 to 32.9	31.3 to 35.6	32.0 to 34.4
CV (%)	6.3% to 8.1%	7.9% to 8.5%	7.3% to 8.4%

The total precision CVs of the Three CsA Lots were generally consistent at all three study sites. For the Level 1 Control, the total precision did not exceed a CV of greater than 19.1 per cent, while for the Level 2 Control, the total precision did not exceed a CV of greater than 10 per cent. Finally, for the Level 3 Control, the total precision did not exceed a CV of greater than 8.5 per cent.

The overall total precision results from the second study were also consistent with the overall total precision results from the first study.

Conclusions from Precision Studies:

Initial studies on combinations of six Reagent lots and three Calibrator lots demonstrated that the Emit® 2000 Cyclosporine Specific Assay provided acceptable within-run and total precision results. The within-run (within-extract) precision based on two cyclosporine levels was less than or equal to 8.8 per cent. The within-run (between-extract) precision based on three cyclosporine levels was less than or equal to 7.1 per cent. The total precision, using concurrent calibration curves, with two cyclosporine levels was less than or equal to 8.6 per cent. Finally, the total precision, using a stored calibration curve, based on two cyclosporine levels was less than or equal to 6.6 per cent.

The Emit® 2000 Cyclosporine Specific Assay demonstrated good overall precision with six different combinations of Reagents and Calibrators when tested at a total of seven clinical study sites. For cyclosporine levels greater than approximately 100 ng/mL, the within-run precision was consistently below 8 per cent CV and total precision was consistently below 12 per cent.

2. Recovery Studies

a. Recovery From Spiked Samples - Transplant Patients

In this study, recovery of cyclosporine spiked into sample pools from individuals undergoing cyclosporine therapy was evaluated. Three whole blood sample pools from patients with each of the three organ transplant types (heart, liver, and kidney) were spiked with two levels of cyclosporine (100 ng/mL and 250 ng/mL). Each cyclosporine-spiked sample pool and the corresponding original unspiked sample pool were assayed. Per cent recovery of the added cyclosporine was determined. Table 6 summarizes the results from the spiked cyclosporine recovery into transplant patient samples.

Table 6
Recovery of Cyclosporine From Spiked Patient Sample Pools

Transplant Type	Pool Number	Baseline Cyclosporine (CsA) Concentration (ng/mL)	% Recovery of Added CsA: 100 ng/mL	% Recovery of Added CsA: 200 ng/mL
Renal	1	146	101	87
Renal	2	153	86	86
Renal	3	125	94	97
Liver	1	228	86	92
Liver	2	200	101	87
Liver	3	197	116	85
Heart	1	244	115	88
Heart	2	231	108	94
Heart	3	93	104	104

The Emit® 2000 Cyclosporine Specific Assay values for the unspiked sample pools ranged from 93 to 244 ng/mL of cyclosporine. Recovery of added cyclosporine ranged from 85 to 116 per cent. All mean recoveries of cyclosporine from patient samples were within 16 per cent of nominal. No trends in recovery across transplant types were evident.

b. Recovery From Spiked Samples - Healthy Donors

Recovery of cyclosporine added to healthy donor blood ranged from 96.1 to 108.1 per cent. All

mean recoveries of the samples were within 8.1 per cent of nominal indicating that cyclosporine could be reproducibly recovered from spiked healthy donor blood.

c. Recovery Upon Dilution of High Samples

There are two optional dilution procedures for sample values above the highest calibrator limit of 500 ng/mL: Pretreated samples may either be diluted with 25 per cent methanol in the assay diluent or, the original untreated sample may be diluted with negative calibrator (Calibrator 0), pretreated, and assayed.

Twelve cyclosporine samples (> 500 ng/mL) were diluted in accordance with the two dilution protocols. The diluted samples were tested with the three CsA Lots and the three COBAS MIRA model instruments. The percent recovery was calculated, and compared the theoretical sample concentrations to the measured concentrations from the samples when diluted in either the Calibrator 0 or the 25 per cent methanolic diluent. Table 7 summarizes the results of the dilution protocols study for the 12 samples.

**Table 7
Dilution Protocols Study:
Summary of Cyclosporine Recovery
from High Cyclosporine Patient Samples (> 500 ng/mL)**

Reagent/Calibrator Lots (One Mira Model)		MIRA Models (Three MIRA Models)	
Calibrator Zero Mean % Recovery Range	25% Methanolic Diluent Mean % Recovery Range	Calibrator Zero Mean % Recovery Range	25% Methanolic Diluent Mean % Recovery Range
98.4% to 101.2%	95.5% to 97.3%	98.7% to 102.1%	96.9% to 99.7%

Conclusions from the Dilution Protocols Studies

The study of the two dilution protocols demonstrated good cyclosporine per cent recoveries. Both dilution protocols provided greater than 95 per cent recoveries with the Three CsA Lots and on all three COBAS MIRA model instruments. These studies demonstrated that the dilution protocols could reliably lower patient samples, with concentrations greater than 500 ng/mL, into the 0 to 500 ng/mL Emit® 2000 Cyclosporine Specific Assay range.

d. Dilution Parallelism

Dilution linearity of high cyclosporine concentration whole blood samples was evaluated using 12 samples (4 heart, 4 kidney, 4 liver) with cyclosporine concentrations > 500 ng/mL. For each sample, a series of dilutions (4 to 5) were made into methanolic diluent, and then tested on the COBAS MIRA and COBAS MIRA S analyzer. The linearity was evaluated and determined

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acceptable following the recommended procedures in the NCCLS guideline (EP6-P) for evaluating linearity.

Conclusions from the Dilution Parallelism Studies:

The Emit® 2000 Cyclosporine Specific Assay demonstrated linear response when cyclosporine samples greater than 500 ng/mL were diluted into the assay range. In addition, these dilution parallelism studies verified the equivalence of two optional dilution protocols (one using the Negative Calibrator, or one with 25 per cent methanolic diluent).

Conclusions from Nonclinical Recovery Studies:

The recovery studies were conducted on cyclosporine spiked samples from healthy donors and transplant patients. The results from the recovery studies demonstrated that the Emit® 2000 Cyclosporine Specific Assay could reproducibly measure cyclosporine spiked into either healthy donors (mean recoveries were within 8 per cent of the nominal spiked cyclosporine concentrations) or blood from transplant patients undergoing cyclosporine therapy (mean recoveries were within 16 per cent of the nominal spiked cyclosporine concentrations). This is acceptable for an assay of this type.

Pretreated samples using the two optional dilution protocols on samples containing cyclosporine concentrations greater than 500 ng/mL also showed that either procedure produced acceptable recoveries with CVs less than 9.1 percent.

3. Sensitivity

The assay sensitivity level was defined as the lowest concentration of cyclosporine that could be statistically distinguished from zero with 95 per cent confidence. For this study 20 aliquots of the negative calibrator were pretreated then assayed. The mean enzymatic rate and standard deviation (SD) of the 20 sample extracts were calculated. This resultant rate was quantitated from the standard curve. All six lots tested showed sensitivity to be less than 40 ng/mL.

Conclusions from Sensitivity Studies:

Based on a definition of assay sensitivity as the lowest concentration of cyclosporine that can be statistically distinguished from zero with 95 per cent confidence, the Emit® 2000 Cyclosporine Specific Assay demonstrated an assay sensitivity of < 40 ng/mL with six Reagent lots. This definition of assay sensitivity is universally acceptable for an analytical parameter.

4. Interference Testing

a. Effect of Anticoagulants

The use of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant is recommended for

blood specimens intended for cyclosporine measurement. The purpose of this study was to determine whether alternative anticoagulants interfered with cyclosporine quantitation in fresh blood specimens in the Emit® 2000 Cyclosporine Specific Assay.

The five anticoagulants examined in this study were tripotassium EDTA (15 mg/10 mL draw), disodium EDTA (10.5 mg/7 mL draw), sodium heparin (143 USP units/10 mL draw), potassium oxalate/sodium fluoride (20 mg and 25 mg, respectively, per 10 mL draw), and sodium citrate (1 mL of 0.129 M/4 mL draw). Tripotassium EDTA served as the control anticoagulant. Blood from two donors was collected into tubes, each containing a different one of the five anticoagulants. Cyclosporine (200 ng/mL) was added to all samples. The samples were then assayed, and recovery of cyclosporine was determined. Table 8 summarizes the results from the anticoagulants study.

Table 8
Recovery of Cyclosporine From Samples Containing Various Anticoagulants

Anticoagulant	Tripotassium EDTA	Disodium EDTA (Control)	Sodium Heparin	Oxalate/Fluoride	Sodium Citrate
Mean Recovery (ng/mL)	208	204	189	201	197
Recovery (%)	104%	102%	94%	100%	98%
SD (ng/mL)	11.0	7.2	8.0	10.7	11.5
CV (%)	5.3%	3.5%	4.2%	5.3%	5.9%

Average recoveries of 200 ng/mL cyclosporine were within 10 per cent of the control and within 6 per cent of nominal cyclosporine concentration for each of the anticoagulants tested. All CVs were less than 6 per cent. These results indicated that the choice of anticoagulant had no significant effect on recovery of cyclosporine from fresh whole blood samples.

b. Endogenous Interference (Spiked samples)

A study of potentially interfering endogenous substances tested concentrations of 40 mg/mL bilirubin, 20 mg/dL uric acid, 3000 mg/dL triglycerides, and 500 mg/dL cholesterol. The test levels for these compounds were based on the NCCLS guideline for interference testing in clinical chemistry, EP7-P (11). Cyclosporine was spiked into whole blood at 85 ng/mL and 425 ng/mL. Stock solutions of each endogenous compound were added to separate aliquots of each of the two cyclosporine-containing samples to yield the respective potentially interfering substances levels. Appropriate controls were prepared for each compound at each cyclosporine level.

The effects of these compounds on the quantitation of cyclosporine in whole blood samples were tested with the three CsA Lots, and evaluated by comparing the mean recovery of each sample to its corresponding mean control value. Recovery of the test samples was calculated based on the control value in terms of percent recovery relative to the control concentration.

The study of potentially interfering endogenous substances had recoveries which were within the following percentages of the control values: bilirubin (3 per cent); uric acid (8 per cent); triglycerides (6 per cent); and cholesterol (11 per cent). These results are consistent with the first study results.

c. Endogenous Interference - Naturally Occurring Levels

Naturally high levels of the endogenous substances were tested for their effect on cyclosporine recovery. The substances examined were bilirubin (> 4 mg/dL), uric acid (> 7 mg/dL), triglycerides (> 190 mg/dL), and cholesterol (> 300 mg/dL). The minimum test levels were set at or above the upper limits of the normal ranges.

Thirteen cyclosporine-free, whole blood samples with high levels of one or more of the test substances were obtained. There were at least three samples with high levels for each substance. Three normal-donor samples were also obtained. Cyclosporine was spiked into the samples at 75 and 425 ng/mL. Mean cyclosporine recovery in the presence of high levels of each test substance was compared to mean recovery from the three normal donor control samples. Table 9 summarizes the study results for naturally high occurring endogenous substances.

**Table 9
Recovery of Cyclosporine from Samples
Containing Naturally High Levels of Endogenous Substances**

Endogenous Substance	Recovery of Cyclosporine (75 ng/mL)	Recovery of Cyclosporine (425 ng/mL)
Normal Donor (n=12)		
Mean Assayed Value (ng/mL)	71	378
Recovery (%)	94.3%	89.1%
CV (%)	10.7%	5.3%
Bilirubin (n=12)		
Mean Assayed Value (ng/mL)	82	411
Recovery (%)	108.7%	96.8%
CV (%)	9.6%	7.3%
Uric Acid (n=16)		
Mean Assayed Value (ng/mL)	80	408
Recovery (%)	106%	96.1%
CV (%)	10.3%	5.4%

Triglycerides (n=16)		
Mean Assayed Value (ng/mL)	85.3	420
Recovery (%)	113.7%	98.8%
CV (%)	8.7%	8.0%
Cholesterol (n=12)		
Mean Assayed Value (ng/mL)	79	387
Recovery (%)	94.3%	91.1%
CV (%)	11.2%	10.6%

Mean recovery of cyclosporine from the 75 ng/mL samples ranged from 94.3 per cent for the normal donor samples to 113.7 per cent for the samples with high levels of triglycerides. The differences in mean recovery of the 425 ng/mL samples ranged from 89.1 per cent for the normal control samples to 98.8 per cent for the samples with high levels of triglycerides. The presence of elevated levels of naturally occurring endogenous substances did not interfere with the measurement of cyclosporine.

d. Hematocrit

In the hematocrit study, fresh whole blood samples with hematocrits varying from approximately 15 per cent to 60 per cent were artificially prepared. Cyclosporine was added to each of the resulting samples at two levels: 85 ng/mL and 425 ng/mL. The samples were extracted and assayed with the three CsA Lots. The mean per cent recovery from each sample was determined. Table 10 summarizes the results of the hematocrit study after testing with the three CsA Lots.

Table 10
Summary of Hematocrit Study:
Effect of Hematocrit on Cyclosporine Recovery

Tested Cyclosporine Level	Percent Recovery (%)	Percent Recovery (%)	Percent Recovery (%)	Percent Recovery (%)	Percent Recovery (%)
	Percentage of Hematocrit				
	15%	26%	37%	48%	60%
85 ng/mL	91 to 97	89 to 96	94 to 106	92 to 103	90 to 98
425 ng/mL	90 to 97	93 to 97	96 to 104	93 to 105	95 to 102

At the 85 ng/mL cyclosporine level, the cyclosporine recoveries ranged from 89 per cent to 106 per cent, and at the 425 ng/mL level, the recoveries ranged from 90 per cent to 105 per cent.

Recoveries at the extremes of hematocrit levels (15 per cent and 60 per cent), deviated less than 10 per cent from those observed with hematocrit values closer to the normal range. The hematocrit study results indicated that the influence of hematocrit on cyclosporine recovery in the Emit® 2000

Cyclosporine Specific Assay was negligible.

d. Cross-Reactivity

i) Cyclosporine Metabolites

Studies were performed to evaluate the performance of the Emit® Emit 2000 Cyclosporine Specific Assay in the presence of four common cyclosporine metabolites: AM9 (M1), AM1 (M17), AM19 (M8), and AM4N (M21).

The substances tested for cross-reactivity in the Emit® 2000 Cyclosporine Specific Assay were tested in the presence of 200 ng/mL cyclosporine on the COBAS MIRA and COBAS MIRA S. Each substance was diluted in an appropriate solvent (water, ethanol, methanol, etc.). A compound was defined as a cross-reactant if it caused a 20 per cent or greater deviation in the apparent cyclosporine (CsA) concentration of that sample in the presence of the metabolite.

The per cent cross-reactivity was determined by the following equation:

$$\frac{\text{Measured CsA Concentration (ng/mL) - Measured Concentration of CsA Control (ng/mL)}}{\text{Cross-Reactant Concentration (ng/mL)}} \times 100$$

(in the presence of metabolite) (nominal value = 200 ng/mL)

No significant cross-reactivity (≤ 10 per cent) was observed for metabolites AM1 (M17), AM9 (M1), AM19 (M8) or AM4N (M21) at levels up to 1000 ng/mL.

A second cross-reactivity study of the same common cyclosporine metabolites as in the first study was performed with the Emit® 2000 Cyclosporine Specific Assay using the COBAS MIRA Plus instrument with the Three CsA Lots. Each metabolite was tested at three concentrations and in the presence of a nominal cyclosporine concentration of 200 ng/mL. A Control sample was also prepared and consisted of the 200 ng/mL cyclosporine concentration in the absence of metabolite. Table 11 summarizes the results of the second cross-reactivity study.

Table 11
Summary of the Second Cross-Reactivity Study:
Percent Cross-Reactivity of Cyclosporine (CsA) Metabolites
in the Presence of 200 ng/mL Cyclosporine

Cyclosporine Metabolite Level (ng/mL)	Metabolite AM1 (M17) % Cross-Reactivity	Metabolite AM9 (M1) % Cross-Reactivity	Metabolite AM4N (M21) % Cross-Reactivity	Metabolite AM19 (M8) % Cross-Reactivity
100	-----	-----	-----	-----
200	-----	3.4% to 10.0%	0.8% to 3.8%	0 to 1.0%
350	-----	-----	-----	-----
500	-----	7.7% to 8.2%	0.2% to 2.6%	-----
600	-----	-----	-----	0 to 1.8%
1000	0 to 2.6%	9.0% to 9.9%	0 to 2.95	0 to 0.5%
1500	0 to 0.9%	-----	-----	-----
2000	0.1% to 1.2%	-----	-----	-----

In the second cyclosporine metabolite cross-reactivity study, no significant cross-reactivity was observed for metabolites AM1 (M17), AM9 (M1), AM19 (M8) or AM4N (M21), at levels up to 1000 ng/mL for AM9, AM4N, AM19, and up to 2000 ng/mL for AM1, respectively. These results are consistent with the first cross-reactivity study.

ii) Co-Administered Drugs

Fifty-seven drugs that are commonly co-administered with cyclosporine were evaluated for cross-reactivity. Test concentrations for the drugs were based on NCCLS guidelines for interference testing. No crossreactivity was observed for any of the drugs at the levels tested in the Emit 2000 Cyclosporine Specific Assay.

- | | |
|----------------|-----------------------------|
| acetaminophen | isoniazid |
| albuterol | isoproterenol hydrochloride |
| allopurinol | ketoconazole |
| alprazolam | lidocaine |
| amitriptyline | lovastatin |
| amphotericin B | methylprednisolone |
| atenolol | metoclopramide |
| azathioprine | misoprostol |
| captopril | morphine sulfate |
| carbamazepine | Muromonab-CD3 |

cefaclor	naproxen
chloramphenicol	nitroglycerin
cimetidine	omeprazole
ciprofloxacin	phenobarbital
cyclophosphamide	phenytoin
digoxin	piperacillin
dipyridamole	prazosin
disopyramide	prednisolone
encainide	prednisone
erythromycin	promethazine
ethanol	ranitidine
fluconazole	salicylic acid
furosemide	sulfamethoxazole
ganciclovir	theophylline
gentamicin	triamterene
heparin	trimethoprim
hydralazine	valproic acid
hydrochlorothiazide	vancomycin
immune globulin	

iii) Other Immunosuppressants

This study was performed to determine the potential cross-reactivity of several immunosuppressants that either have been recently approved by the FDA, or are being used in clinical research studies. The following 4 compounds were tested using the three CsA Lots and the MIRA Plus analyzer:

- FK506 (tacrolimus)
- MPA (mycophenolic acid, active drug from the prodrug, mycophenolic acid mofetil)
- MPAG (MPA-glucuronide, a metabolite of MPA)
- Rapamycin

Each compound was tested in the presence of a nominal concentration of 200 ng/mL of cyclosporine. The levels of the compounds tested were much greater than the therapeutic levels. Immunosuppressant samples and their corresponding control sample (no immunosuppressant present) were assayed. Recovery was measured as percentage of a control value.

Table 12 summarizes the Immunosuppressant Study using the three CsA Lots.

Table 12
Summary of Percent Cross-Reactivity
of Four Immunosuppressant Compounds

Compound	Concentration	Sample Range	% Cross Reactivity
FK506	0.1	208 to 277	1.6% to 4.2%
MPA	50	211 to 218	0%
	100	213 to 216	0%
MPAG	500	205-220	0%
	1,000	190 to 222	0%
Rapamycin	0.1	206 to 220	2.8% to 4.6%

For FK506, the range of per cent cross-reactivity was from 1.6 per cent to 4.2 per cent. For MPA, and its metabolite MPAG, there was no cross-reactivity observed. For Rapamycin, the range of per cent cross-reactivity was from 2.8 per cent to 4.6 per cent. For the four immunosuppressants, at all levels tested, the Emit® 2000 Cyclosporine Specific Assay provided a maximum cross-reactivity of less than 5 per cent.

Conclusions from Potentially Interfering Substances Studies:

Studies were conducted to determine if the performance of the Emit® 2000 Cyclosporine Specific Assay would be affected by: the use of different anticoagulants; potentially interfering endogenous substances; hematocrits, or cross-reacting substances (common cyclosporine metabolites, co-administered drugs, other immunosuppressants).

- a) The performance of the Emit® 2000 Cyclosporine Specific Assay was not affected by the use of the following anticoagulants: tripotassium EDTA, disodium EDTA, sodium heparin, potassium oxalate/sodium fluoride, and sodium citrate. Mean recoveries of cyclosporine were within 6 per cent of the nominal cyclosporine concentration and demonstrated CVs of less 6 per cent.

b) Various levels of potentially interfering endogenous substances were studied to determine if they had any effect on the performance of the Emit® 2000 Cyclosporine Specific Assay. When these substances (bilirubin, uric acid, triglycerides, cholesterol) were either spiked at artificially high levels into normal donor whole blood samples, or tested at naturally occurring high levels, the performance of the Emit® 2000 Cyclosporine Specific Assay was not affected. Cyclosporine recoveries of samples artificially spiked with cyclosporine were within 3 per cent to 11 per cent of the control levels, depending on the substance. The cyclosporine recoveries (two levels) of samples with naturally occurring high levels of potentially interfering substances were higher than with the spiked samples, but still acceptable at ≤ 14 per cent for the low cyclosporine level, and ≤ 11 per cent for the high cyclosporine level.

c) The performance of the Emit® 2000 Cyclosporine Specific Assay was not significantly affected by different levels of hematocrit.

d) The Emit® 2000 Cyclosporine Specific Assay did not demonstrate cross-reactivity above 10 per cent with four common cyclosporine metabolites: AM1 (M17), AM9 (M1), AM19 (M8) and AM4N (M21), at levels up to 1000 ng/mL.

e) Fifty-seven drugs that are commonly co-administered with cyclosporine, also did not demonstrate any significant cross-reactivity in the Emit® 2000 Cyclosporine Specific Assay.

f) Finally, four immunosuppressant drugs (either legally marketed or used in clinical research studies): FK506, MPA, MPAG, and Rapamycin were tested for their ability to interfere with the performance of the Emit® 2000 Cyclosporine Specific Assay. The study results showed that a maximum of 5 per cent cross-reactivity was observed with the Emit® 2000 Cyclosporine Specific Assay, and therefore would not significantly affect the performance of the assay.

5. Other Characteristics

a. Carryover

Samples were pretreated then assayed in a prescribed pattern. The samples were placed in a series which consisted of one high cyclosporine sample (> 210 ng/mL) followed by two samples of the Level 1 Controls (approximate cyclosporine concentration 70 ng/mL). The per cent carryover from each of the test samples into the Level 1 Control was estimated by calculating the per cent carryover from each individual test sample, and then determining the overall mean for each test sample level.

Four high cyclosporine samples (ranging from 210 ng/mL to 800 ng/mL) were tested in this manner. Three Emit® 2000 Cyclosporine Specific Assay Reagent/Calibrator combination lots were used in this study. Table 13 summarizes the first carryover study results.

Table 13
Summary of First Carryover Study for Four Cyclosporine Levels

Cyclosporine Spike Level (mg/mL)	Range of Estimated Per Cent Carryover
210 ng/mL	1.6% to 2.9%
370 ng/mL	2.1% to 4.0%
500 ng/mL	2.1 % to 2.9%
800 ng/mL	1.9% to 2.7%

The estimated per cent carryover into the Level 1 Control from samples with concentrations ranging from 500 to 800 ng/mL was less than 3 per cent. This was equivalent to less than 24 ng/mL of cyclosporine carryover from an 800 ng/mL sample. This cyclosporine level was less than the assay sensitivity (lower limit of detection) of the Emit® 2000 Cyclosporine Specific Assay.

Some variability in the level of carryover and in the effect of sample carryover on precision was noted among the different pipetter-diluters and MIRA analyzers used in these studies.

In a second carryover study, three Reagents/Calibrators combination lots were used. Four high cyclosporine samples (Level 1 control of 70 ng/mL and samples of 400 ng/mL, 500 ng/mL and 800 ng/mL) were tested in this manner. Three Emit® 2000 Cyclosporine Specific Assay Reagent/Calibrator combination lots were used in this study. Table 14 summarizes the second carryover study results.

Table 14
Summary of Second Carryover Study for Three Cyclosporine Levels

Cyclosporine Spike Level (ng/mL)	Range of Estimated Per Cent Carryover
400 ng/mL	0.4% to 3.3%
500 ng/mL	0.9% to 2.9%
800 ng/mL	0.5% to 1.8%

The results indicated that over the range of tested cyclosporine levels (400 ng/mL to 800 ng/mL), the maximum mean carryover observed was ≤ 3.3 per cent which is consistent with the first carryover study results, and continues to translate to cyclosporine levels below the sensitivity of the assay.

The carryover study was repeated with all three COBAS MIRA model instruments (MIRA, MIRA S, MIRA Plus). Across all three models, the estimated per cent carryover observed ranged between 0.1 per cent to 3.3 per cent which is consistent with the previously described carryover study.

Conclusions from Carryover Studies:

The results from the studies showed that the Emit® 2000 Cyclosporine Specific Assay demonstrated an overall estimated carryover 4 per cent or less, which translates to a cyclosporine concentration

which is less than the assay's sensitivity (limit of detection). Therefore, sample carryover is unlikely to have a significant effect on patient sample quantitation either in terms of accuracy or precision in the routine use of the Emit® 2000 Cyclosporine Specific Assay.

b. Proficiency Survey Participation

Samples used in two proficiency surveys were assayed by the Emit® Emit 2000 Cyclosporine Specific Assay: The American Association of Clinical Chemists/College of American Pathologists (AACC/CAP), and The United Kingdom Quality Assessment Scheme (UKQAS).

The AACC/CAP included samples which were spikes of cyclosporine only, while the UKQAS survey was more comprehensive. Most often, UKQAS samples were patient sample pools, but they can also be a cyclosporine-negative sample matrix spiked with either cyclosporine or metabolites or a combination of both. Results by the Emit® 2000 Cyclosporine Specific Assay were compared to two commercial immunoassay methods, for which there are approved PMAs, and to reference HPLC procedures. The correlation between Emit versus one of the commercial methods (the Reference method) was 0.985, while the correlation between Emit versus the other commercial method was 0.993. The correlation between Emit versus HPLC procedures was 0.945.

Conclusions from the Proficiency Survey Participation:

The results of two different proficiency surveys demonstrated that the Emit® 2000 Cyclosporine Specific Assay performed similarly to three other assay methods for measuring cyclosporine. The correlations between the Emit® 2000 Cyclosporine Specific Assay and the other methods ranged from 0.945 to 0.993.

c. Lot-to-Lot Interchangeability of Reagents and Calibrators

The Emit® 2000 Cyclosporine Specific Assay Reagents and Calibrators are intended to be sold separately (i.e., any set of matched Reagents may be used with any set of Calibrators). With this in mind, crossover studies were performed between the three assay Reagent lots and the three Calibrator lots. The results from each of the nine combinations were evaluated.

Each of three different sets of three of the nine Reagents/Calibrators lot combinations were tested in single runs on a MIRA Plus (#1) analyzer. Each run included the testing of five samples (a test panel) containing a range of cyclosporine concentrations. The nominal concentrations of cyclosporine in the test panel of five samples (Levels 1 to 5) were: 85 ng/mL, 170 ng/mL, 255 ng/mL, 340 ng/mL, and 425 ng/mL, respectively. The means and CVs were calculated for each of the five levels using all nine Reagents/Calibrators lot combinations.

Table 15 presents the mean CsA values and the CVs for each Reagent/Calibrator combination from the Lot-to-Lot Interchangeability of Reagents and Calibrators Study.

Table 15
Lot-to Lot Interchangeability of Reagents and Calibrators Study

CsA Level	Reagent Lot	Calibrator Lot Y3 Mean CsA (%CV)	Calibrator Lot Y4 Mean CsA (%CV)	Calibrator Lot Y5 Mean CsA (%CV)
1	Y2	85.2 (6.6%)	84.9 (8.2%)	84.0 (8.0%)
1	Y3	79.8 (8.1%)	84.1 (9.3%)	83.8 (7.3%)
1	Y4	81.5 (3.2%)	81.4 (4.4%)	81.2 (8.7%)
2	Y2	170.9 (3.8%)	161.8 (4.4%)	162.5 (3.6%)
2	Y3	165.9 (2.9%)	167.8 (5.8%)	163.0 (2.8%)
2	Y4	171.7 (2.2%)	168.7 (5.4%)	164.5 (3.0%)
3	Y2	263.1 (2.0%)	245.8 (4.1%)	249.6 (2.6%)
3	Y3	253.3 (2.2%)	257.2 (4.2%)	250.8 (2.5%)
3	Y4	259.2 (3.3%)	256.4 (3.0%)	243.3 (3.0%)
4	Y2	345.9 (4.1%)	326.4 (3.3%)	323.5 (3.2%)
4	Y3	339.0 (2.0%)	330.0 (3.8%)	328.9 (1.6%)
4	Y4	347.0 (3.6%)	331.8 (3.8%)	334.9 (2.3%)
5	Y2	422.3 (3.8%)	396.9 (5.1%)	382.1 (2.4%)
5	Y3	402.0 (3.6%)	388.5 (3.6%)	384.5 (5.2%)
5	Y4	408.6 (5.3%)	393.6 (3.1%)	388.8 (3.4%)

There was no combination of Reagent and Calibrator lots where the differences in the mean cyclosporine values of any of the test panel members was greater than 10 per cent when compared to any other combination. None of the nine combinations, at any Level, yielded mean CV values above 10 per cent.

Conclusions from Lot-to-Lot Interchangeability of Reagents and Calibrators Study:

There were no significant differences between the nine Reagent/Calibrator combinations in terms of mean cyclosporine results or CV. All nine Reagent/Calibrator combinations provided acceptable mean cyclosporine results and CV values at all 5 cyclosporine levels tested.

B. Clinical Studies

1. Objectives

The overall objective was to demonstrate that the Emit® 2000 Cyclosporine Specific Assay was safe and effective for its intended use. This was done in part by comparing the accuracy of results of the Emit® 2000 Cyclosporine Specific Assay with those of a commercial RIA-based specific assay (referred to as the Reference assay), for which there is an approved PMA, during serial monitoring of patients after kidney, heart, or liver transplants. Results from individual patients and combined patient data were analyzed with respect to consistency among organ types and transplant centers. For the same patient population, the results of the Emit® 2000 Cyclosporine Specific Assay (Emit) were also compared with those of the HPLC procedures.

2. Study Sites

The first clinical study was conducted at four transplantation centers:

- University of Alabama at Birmingham Medical Center (U. Alabama)
- University of Cincinnati Medical Center (U. Cincinnati)
- University of Washington Medical Center (U. Washington)
- The Mayo Clinic (Mayo).

Three centers performed CsA analyses using the Emit and the Reference assay as well as by the HPLC procedures. A fourth center (Mayo) performed cyclosporine analyses by the Emit assay and by the HPLC procedures, and sent the samples to another site for analysis by the same Reference assay.

The second clinical study was conducted at three transplantation centers:

- University of Alberta (UA)
- University of California at Los Angeles, School of Medicine (UCLA)
- University of Washington Medical Center (UW)

All clinical treatment followed the established protocol of the particular institution. All samples for use in these studies were taken during the normal course of treatment (no additional samples were drawn).

3. Study Population

First Clinical Study

One hundred and thirty-six transplant patients began the study: 30 heart patients, 64 kidney patients, and 42 liver patients. Eighty-six percent of the patients completed the study as planned (117). In total, over 4,000 blood samples were analyzed by at least two of the three test methods. The patients were followed for an average of 127 days, or 4.2 months. The range was 37 to 245 days.

Demographic data was obtained for 128 patients (51 females and 77 males). The average age of patients in this study was approximately 44 years, with a range from 4 years to 71 years. One patient was 4 years old, two were 13 years old, and the rest were 16 years and older. Fourteen patients were 60 years old or older. Most of the patients were Caucasian (105), with 21 Black, 1 Asian, and 1 Hispanic.

Second Clinical Study

A total of 132 fresh patient samples were assayed using the Three CsA Lots at all external sites (UA, UCLA, and UW). The samples were selected to: span the Emit[®] 2000 Cyclosporine Specific Assay range (40 ng/mL to 500 ng/mL) based on their HPLC values (as determined at UW), to represent the three transplant types (50 liver, 35 kidney, 47 heart), and to encompass varying times post-transplant (range: 3 to 2499 days, median 111 days).

4. Data Analysis

Method comparisons were performed on combined patient data (all clinical studies) and on the patients' individual statistical data (first clinical studies).

a. Combined Patient Data

The three test methods used in the first study were compared by combining measurements from all patients at each site. Assay comparisons were based on linear regressions of blood sample measurements from all patients (i.e., data points from individual patients were combined by site, method, or organ.).

For the first clinical study, the combined patient data results were obtained from the three test methods performed on approximately 4,000 patient samples at four centers.

All cyclosporine blood level results from the first clinical study were included in the analysis of this section. That is, patients excluded from the individual statistical analysis due to insufficient numbers of samples were included here

b. Individual Patient Analysis

Results from the three test methods based on analysis of individual patients' summary data were compared. The summary for each patient's three pairs of results (i.e., Emit versus Reference, Emit versus the HPLC procedure, and Reference versus HPLC procedure) was in the form of a Pearson's correlation coefficient. For each pair of assay results, the slope and intercept from an orthogonal (or Deming) regression were estimated for each patient. Data gathered during episodes of rejection or nephrotoxicity were described.

For each test method comparison an analysis of variance (ANOVA) was used to test for the effect of study site and organ type on the correlation between the methods. This analysis included only the study sites that tested all three organ types. The CVs, and the slopes and intercepts from the orthogonal regressions were then summarized for all patients. These were further summarized separately for each study site

Results of Clinical Studies

1. Combined Patient Data

Linear regression results of method comparisons separated by organ type at each center were analyzed. The slopes for the Emit (y-axis) versus the Reference (RIA) assay (x-axis) across study centers and organ types ranged from 0.79 to 1.04. The slopes for Emit (y-axis) versus HPLC procedures (x-axis) ranged from 0.88 to 1.26, and the slopes for HPLC procedures (x-axis) versus RIA (y-axis) ranged from 1.04 to 1.49.

The correlation coefficient values (R values) for the Emit versus the Reference method ranged from 0.915 to 0.978. The R values for the Emit versus the HPLC procedures ranged from 0.773 to 0.968; while for HPLC procedures versus RIA, the values ranged from 0.759 to 0.973.

A clinical study was performed with the Emit® Emit 2000 Cyclosporine Specific Assay in which 138 patient samples (50 liver, 37 kidney, 51 heart) were tested at three study sites (UA, UCLA, UW) with three Reagents/Calibrators combination lots. The Emit results were compared to the HPLC procedures results by performing linear regression on the data from each Emit lot. Slope was estimated using Deming (Orthogonal) regression.

Conclusions from the Analysis of Combined Patient Data:

Overall, the slopes and R values from the linear regression analyses of the combined patient data indicated that the Emit® 2000 Cyclosporine Specific Assay compared the closest with the Reference (RIA) method. The ranges demonstrated across these comparisons have also been observed with other cyclosporine devices for which there are approved PMAs.

2. Individual Patient Statistics

a. Emit Versus Reference Assay

The purpose of this analysis was to assess the agreement between the Emit assay results and the Reference (RIA) assay results, and to test to see if this agreement was consistent among organ types and among four study sites. One hundred and seventeen patients met the minimum criteria for inclusion in this analysis (26 heart, 58 kidney, 33 liver). Table 16 presents a summary of the Emit versus the Reference (RIA) method results for each study site.

Table 16
Emit (Y-Axis) Versus Reference (X-Axis) Results
Summary of Three Organs Types at Four Study Sites

Organ Type	Number of Samples	Number of Patients	Mean Correlation Coefficients (95% CI)	Mean Slope (95% CI)
U. of Alabama				
Heart	377	10	0.93 (0.90 to 0.96)	0.82 (0.70 to 0.94)
Kidney	442	17	0.94 (0.92 to 0.96)	0.75 (0.67 to 0.84)
Liver	373	10	0.91 (0.88 to 0.94)	1.00 (0.85 to 1.16)
U. of Cincinnati				
Heart	169	6	0.89 (0.81 to 0.97)	0.79 (0.68 to 0.89)
Kidney	365	12	0.93 (0.91 to 0.95)	0.82 (0.78 to 0.86)
Liver	211	6	0.90 (0.78 to 1.00)	0.81 (0.74 to 0.89)
U. of Washington				
Heart	503	10	0.98 (0.97 to 0.98)	0.81 (0.79 to 0.84)
Kidney	579	18	0.96 (0.95 to 0.98)	0.87 (0.83 to 0.91)
Liver	491	10	0.97 (0.97 to 0.98)	0.93 (0.90 to 0.96)
Mayo Clinic				
Heart	*	*	N/A	N/A
Kidney	330	11	0.96 (0.95 to 0.97)	0.84 (0.77 to 0.92)
Liver	252	7	0.95 (0.91 to 0.99)	0.78 (0.71 to 0.84)

*No heart transplants were performed at the Mayo Clinic.

Among the three study sites which collected data for all three organ types (Mayo Clinic did not perform any heart transplants during the study), there was a statistically significant difference in R values among study sites ($p < 0.01$) across organ types. The University of Washington had the highest mean R value at 0.97. The mean R values for the remaining two sites were 0.93 and 0.91 for the University of Alabama and University of Cincinnati, respectively.

The R values were not statistically significant among organ types ($p = 0.24$) at each site. This indicated that the agreement between Emit and the Reference results was consistent among organ types.

ANOVA analysis of the three sites which collected data on all three organ types revealed that there was a statistically significant difference ($p < 0.01$) in slopes (Emit, y-axis vs. Reference, x-axis) among organ types at two study sites (University of Alabama and University of Washington). The University of Cincinnati did not demonstrate any statistically significant difference in slopes among organ types ($p = 0.61$).

Across all study sites, for each organ type, the mean R values were 0.94 for heart patients and liver patients, and 0.95 for kidney patients. The slopes (Emit, y-axis vs. Reference, x-axis), across study sites, among organ types were more variable than the R values. The mean slopes, across all sites, for each organ type, were slightly less than 1.0. The mean slopes were 0.81 for heart patients, 0.82 for kidney patients, and 0.90 for liver patients.

Conclusions from the Analysis of Emit Versus Reference Assay Results:

The Emit® 2000 Cyclosporine Specific Assay demonstrated acceptable agreement with the Reference Assay. While there was a statistically significant difference between the two methods, there was no statistically significant difference between organ types and the two methods.

b. Emit Versus HPLC Procedures

The purpose of this analysis was to assess the agreement between the Emit assay results and the HPLC procedures results, and to test to see if this agreement was consistent among organ types and among study sites. One hundred and twenty-eight patients met the minimum criteria for inclusion in this analysis (30 heart, 62 kidney, 36 liver). Table 17 presents a summary of the Emit versus HPLC procedures results for each study site.

Table 17

**Emit (Y-Axis) Versus HPLC Procedures (X-Axis) Results
Summary of Three Organs Types at Four Study Sites**

Organ Type	Number of Samples	Number of Patients	Mean Correlation Coefficients (95% CI)	Mean Slope (95% CI)
U. of Alabama				
Heart	348	10	0.92 (0.89 to 0.94)	1.22 (1.09 to 1.34)
Kidney	432	17	0.86 (0.81 to 0.92)	1.12 (0.98 to 1.25)
Liver	357	10	0.87 (0.79 to 0.94)	1.12 (1.06 to 1.18)
U. of Cincinnati				
Heart	243	10	0.80 (0.70 to 0.89)	0.88 (0.70 to 1.07)
Kidney	494	16	0.87 (0.83 to 0.90)	0.93 (0.80 to 1.07)
Liver	266	9	0.78 (0.64 to 0.93)	0.92 (0.71 to 1.14)
U. of Washington				
Heart	501	10	0.97 (0.96 to 0.98)	1.03 (0.98 to 1.09)
Kidney	579	18	0.96 (0.94 to 0.97)	1.09 (1.04 to 1.14)
Liver	490	10	0.96 (0.95 to 0.98)	1.08 (1.01 to 1.15)
Mayo Clinic				
Heart	*	*	N/A	N/A
Kidney	327	11	0.95 (0.92 to 0.97)	1.07 (0.97 to 1.16)
Liver	252	7	0.94 (0.90 to 0.98)	0.95 (0.85 to 1.06)

*No heart transplants were performed at the Mayo Clinic.

Among the three study sites which collected data on all three organ types (Mayo Clinic did not perform any heart transplants during the study), there was a statistically significant difference in R values among study sites ($p < 0.01$). The University of Washington had the highest mean R values. The mean R values, over all organ types, were 0.88, 0.81, and 0.96 for the Universities of Alabama, Cincinnati, and Washington, respectively.

The R values were not statistically significant among organ types ($p = 0.46$) indicating that the agreement between Emit and the HPLC procedures results was consistent among organ types.

ANOVA analysis of the three sites which collected data on all three organ types (Mayo Clinic did not perform any heart transplants during the study) revealed that there was no statistically significant difference ($p = 0.99$) in slopes among organ types.

A statistically significant difference ($p < 0.01$) was found for slopes (Emit, y-axis vs. HPLC procedures, x-axis) across study sites. At the University of Alabama, the mean slope across organ types was 1.15. At the University of Cincinnati, this mean was 0.91, while at the University of Washington, the mean slope across organ types was 1.07

Across all study sites, for each organ type, the mean R values were 0.89 for heart patients and liver patients, and 0.91 for kidney patients. The mean slopes, across study sites, among organ types (which did not show any statistically significant difference) were 1.04 for heart patients, 1.05 for kidney patients, and 1.03 for liver patients.

Conclusions from the Analysis of Emit Versus HPLC Procedures Results:

The Emit® 2000 Cyclosporine Specific Assay demonstrated good agreement with the HPLC procedures. There was no statistically significant difference between sites among organ types with the overall mean R values for each organ type, across sites, ranging from 0.89 to 0.91, and the overall mean slopes for each organ type ranging from 1.03 to 1.04. These results were similar to the comparison with the reference method.

c. Reference Method Versus HPLC Procedures

The purpose of this analysis was to assess the agreement between the Reference assay results and the HPLC procedures results, and to test to see if this agreement was consistent among organ types and among study sites. One hundred and fourteen patients met the minimum criteria for inclusion in this analysis (26 heart, 56 kidney, 32 liver). Table 18 presents a summary of the Reference (RIA) Method versus HPLC procedures results for each study site.

Table 18

**Reference Method (Y-Axis) Versus HPLC Procedures (X-Axis) Results
Summary of Three Organs Types at Four Study Sites**

Organ Type	Number of Samples	Number of Patients	Mean Correlation Coefficients (95% CI)	Mean Slope (95% CI)
U. of Alabama				
Heart	351	10	0.90 (0.86 to 0.94)	1.52 (1.34 to 1.70)
Kidney	432	17	0.85 (0.79 to 0.91)	1.50 (1.34 to 1.65)
Liver	360	10	0.88 (0.82 to 0.94)	1.15 (1.00 to 1.30)
U. of Cincinnati				
Heart	146	6	0.73 (0.61 to 0.86)	1.11 (0.72 to 1.50)
Kidney	288	10	0.80 (0.72 to 0.87)	1.12 (0.81 to 1.42)
Liver	152	5	0.78 (0.60 to 0.95)	1.13 (0.69 to 1.56)
U. of Washington				
Heart	516	10	0.98 (0.97 to 0.98)	1.27 (1.20 to 1.34)
Kidney	590	18	0.95 (0.93 to 0.98)	1.27 (1.19 to 1.35)
Liver	497	10	0.96 (0.95 to 0.97)	1.16 (1.09 to 1.23)
Mayo Clinic				
Heart	*	*	N/A	N/A
Kidney	327	11	0.96 (0.94 to 0.97)	1.28 (1.21 to 1.34)
Liver	255	7	0.97 (0.94 to 0.99)	1.22 (1.14 to 1.30)

*No heart transplants were performed at the Mayo Clinic.

Among the three study sites which collected data on all three organ types), there was a statistically significant difference in R values among study sites ($p < 0.01$). The University of Washington had the highest mean R values. The mean R values, over all organ types, were 0.88, 0.77, and 0.96 for the Universities of Alabama, Cincinnati, and Washington, respectively. The R values were not statistically significant among organ types ($p = 0.92$) indicating that the agreement between the Reference Method and the HPLC procedure results was consistent among organ types.

ANOVA analysis of the three sites which collected data on all three organ types (Mayo Clinic did not perform any heart transplants during the study) revealed that there was no statistically significant difference ($p = 0.06$) in slopes among organ types.

A statistically significant difference ($p < 0.01$) was found for slopes (Reference, y-axis vs. HPLC procedures, x-axis) across study sites. At the University of Alabama, the mean slope across organ types was 1.39. At the University of Cincinnati, this mean was 1.12, while at the University of Washington, the mean slope across organ types was 1.23.

Across all study sites, for each organ type, the mean R values were 0.89 for heart patients and kidney patients, and 0.91 for liver patients. The mean slopes, across study sites, among organ types (which

did not show any statistically significant difference) were 1.33 for heart patients, 1.31 for kidney patients, and 1.16 for liver patients. These differences are acceptable for devices of this type.

Conclusions from the Analysis of the Reference Method Versus HPLC Procedures Results:

The agreement between the Reference Method and HPLC procedures was similar to the agreement between Emit® 2000 Cyclosporine Specific Assay and HPLC. These studies showed that the reference method and the HPLC method also demonstrated statistical differences across sites, but not among organ types.

Final Conclusions from the Analysis of Individual Patient Statistics:

Based on the analysis of the individual patient statistics, the Emit® Emit 2000 Cyclosporine Specific Assay agreed with the Reference (RIA) method (R value ranges for the three organ types were between 0.94 to 0.95). The comparison of the Emit® 2000 Cyclosporine Specific Assay with the HPLC procedures was similar to the comparison between the Reference method and HPLC procedures (in both cases, the R value ranges for the three organ types were between 0.89 to 0.91).

6. Correlation of Cyclosporine Levels with Serum Creatinine Levels and with Adverse Events

Data was collected by the Emit® 2000 Cyclosporine Specific Assay, the Reference assay, and the HPLC procedures to compare the correlation between: cyclosporine levels and serum creatinine levels; cyclosporine levels and episodes of organ rejection; and cyclosporine levels and episodes of nephrotoxicity.

a. Serum Creatinine Levels

Nephrotoxicity is manifested by a rise in serum creatinine levels. Cyclosporine measurements by the Emit® 2000 Cyclosporine Specific Assay, by the Reference method, and by the HPLC procedures were analyzed from heart, kidney, and liver transplant samples with either normal levels of serum creatinine (less than or equal to 1.7 mg/dL) or abnormal levels of serum creatinine (> 1.7 mg/dL). All cyclosporine measurements that coincided with a serum creatinine measurement were included. For each assay method, for all three organ types, the cyclosporine measurement was always higher in samples with normal levels of serum creatinine than in samples with abnormal (high) levels.

b. Episodes of Organ Rejection

Clinical response to cyclosporine treatment does not correlate well with the administered dose. Thirty-four patients had an episode of organ rejection of at least moderate severity (12 heart, 16 kidney, and 6 liver), according to the assessment of the patients' attending physicians. Patients with cases of rejection that were classified as mild were excluded from this analysis.

In both heart and kidney transplant recipients, cyclosporine levels measured by the Emit® Emit 2000 Cyclosporine Specific Assay tended to be lower in patients with rejection compared to those with no rejection, suggesting under-immunosuppression. No trends could be discerned between the two groups in liver transplant recipients. However, the number of cases of liver allograft rejection was low and the distribution was uneven (3 in the first week after transplant and 3 in the third month post-transplant).

c. Episodes of Nephrotoxicity

Twenty-three patients had at least one episode of nephrotoxicity, according to the assessment of the patients' attending physicians. The distribution among transplant types was relatively even: 7 heart, 8 kidney, and 8 liver. Episodes of nephrotoxicity that occurred in the second and third month post-transplant generally were accompanied by higher Emit cyclosporine levels than episodes that occurred in the first month post-transplant. These later episodes (months 2 and 3) usually had mean Emit cyclosporine levels that were much higher than those seen in the patients with no nephrotoxicity, as described in the Expected Values section, Table 21.

Conclusions from Comparing Cyclosporine Levels with Serum Creatinine Levels and Adverse Events:

In comparing cyclosporine levels with serum creatinine levels, higher cyclosporine levels were measured in normal serum creatinine patients compared to those with abnormally high serum creatinine samples. This is contrary to what would be expected since nephrotoxicity is associated with increased levels of serum creatinine and high levels of cyclosporine.

For two organ transplant types, heart and kidney, the Emit® 2000 Cyclosporine Specific Assay results from patients with rejection episodes, tended to be lower than from patients showing no rejection episodes. For liver patients, the results were inconclusive with no discernible pattern detected between cyclosporine levels and episodes of rejection.

For episodes of nephrotoxicity for the three organ types, during the second and third months post-transplantation, the Emit® 2000 Cyclosporine Specific Assay values tended to be much higher than for samples from patients, during the same time period, having no episodes of nephrotoxicity. This trend was much less apparent for episodes of nephrotoxicity which occurred during the first month of post-transplantation.

Absorption of cyclosporine is highly variable and clearance varies greatly among patients. Factors affecting cyclosporine concentrations in blood include the nature of the transplant, the age of the patient, the general health of the patient (specifically the presence or absence of liver disease or gastrointestinal dysfunction), the patient's lipoprotein profile and hematocrit, and the coadministration of cyclosporine with food or with certain drugs (3).

Conclusion of Studies

Nonclinical Studies

Nonclinical Studies Conclusions were based on using the Emit® 2000 Cyclosporine Specific Assay and reviewing performance in terms of its: precision, recovery of cyclosporine in patient samples, assay sensitivity, effects of potentially interfering substances, and the amount of carryover exhibited by the assay.

Initial nonclinical precision studies demonstrated that the Emit® 2000 Cyclosporine Specific Assay provided acceptable within-run and total precision results. In the second Precision Studies, within-run and total precision tended to be less than 8 per cent for all three COBAS MIRA model instruments.

Cyclosporine recovery studies performed with the Emit® 2000 Cyclosporine Specific Assay showed that the assay could reproducibly measure cyclosporine which was spiked into either healthy blood donors (mean recoveries within 8 per cent of nominal cyclosporine targets) or transplant patients (mean recoveries within 16 per cent of nominal cyclosporine targets). In addition, the Emit® 2000 Cyclosporine Specific Assay demonstrated that the cyclosporine recoveries were linear over the assay's calibration range of 0 to 500 ng/mL.

Cyclosporine recovery studies with the Emit® 2000 Cyclosporine Specific Assay also showed that acceptable performance could be obtained with the assay when using two different dilution protocols for diluting patient samples with cyclosporine levels greater than 500 ng/mL. High cyclosporine recoveries were obtained in two studies (both studies had greater than 90 per cent recoveries).

The Emit® 2000 Cyclosporine Specific Assay sensitivity was demonstrated to be less than 40 ng/mL.

Studies with potentially interfering substances demonstrated that the Emit® 2000 Cyclosporine Specific Assay was not affected by any of these substances. Five different anticoagulants were tested with the Emit® 2000 Cyclosporine Specific Assay. Mean recoveries of cyclosporine were within 6 per cent of the nominal cyclosporine targets for all anticoagulants tested. Abnormally high levels of potentially interfering endogenous substances (bilirubin, uric acid, triglycerides, cholesterol) were studied with the Emit® 2000 Cyclosporine Specific Assay, and were found not to significantly affect the performance of the assay. In artificially spiked samples, the cyclosporine recoveries were within 2 per cent to 11 per cent of the control levels, depending on the substance. In naturally occurring potentially interfering samples, the cyclosporine recoveries were higher, but still acceptable at less than or equal to 14 per cent of the control levels.

Blood hematocrit levels ranging from 15 per cent to 60 per cent did not affect the performance of the Emit® 2000 Cyclosporine Specific Assay. Four common cyclosporine metabolites at levels of up to 1,000 ng/mL, also did not interfere with the performance of the Emit® 2000 Cyclosporine Specific Assay. In addition, 57 drugs which are commonly co-administered with cyclosporine, also did not

affect the performance of the Emit® 2000 Cyclosporine Specific Assay. A study also demonstrated that four currently used immunosuppressant drugs did not affect the performance of the Emit® 2000 Cyclosporine Specific Assay

When the Emit® 2000 Cyclosporine Specific Assay was analyzed for the amount of potential carryover exhibited by the assay at several cyclosporine levels, it was estimated that a maximum of 3 per cent carryover was present. This percent carryover translated to an amount of cyclosporine which was less than the assay's sensitivity. Therefore, the amount of carryover exhibited by the Emit® 2000 Cyclosporine Specific Assay was clinically insignificant.

Dilution parallelism studies were performed with the Emit® 2000 Cyclosporine Specific Assay using 12 clinical samples with cyclosporine concentrations greater than 500 ng/mL. A series of dilutions (4 to 5 dilutions per sample) were made for each sample and tested on the MIRA and MIRA S analyzers. Linearity of the results was evaluated based on the NCCLS guideline EP6-P. Linearity was demonstrated for 11 samples (1 borderline linear) on the MIRA instrument. On the MIRA S instrument, linearity was demonstrated for all 12 samples

An analysis of Emit® 2000 Cyclosporine Specific Assay runs using run validation criteria based on tri-level versus bi-level controls, indicated that bi-level controls could be used in place of the tri-level controls. Therefore, the performance of the Emit® 2000 Cyclosporine Specific Assay would not be affected by switching to bi-level controls.

Clinical Studies

Clinical Studies Conclusions were based on using the Emit® 2000 Cyclosporine Specific Assay and reviewing its performance on over 4,000 blood samples which were taken from 136 transplant patients (30 heart, 64 kidney, 42 liver) at four organ transplant centers. From the Clinical Studies, individual patient and combined organ data were analyzed and compared with a Reference RIA method and a HPLC procedure. In addition, the Emit® 2000 Cyclosporine Specific Assay was used to compare correlation of cyclosporine levels with serum creatinine levels and adverse events (rejection and nephrotoxicity episodes).

Analysis of combined patient (organ) data from four clinical trial sites showed that the Emit® 2000 Cyclosporine Specific Assay compared with the Reference (RIA) method. R values for the Emit versus Reference method, across organ types, ranged from 0.92 to 0.98.

A similar analysis of individual patient statistics revealed that the Emit® 2000 Cyclosporine Specific Assay agreed with the Reference (RIA) method with R value ranges across organ types of between 0.94 to 0.95.

No statistical relationship between Emit® 2000 Cyclosporine Specific Assay values with patient serum creatinine levels could be demonstrated. High serum creatinine levels associated with nephrotoxicity were not observed in patients with higher cyclosporine levels. More commonly high cyclosporine

levels were observed in patients with normal creatinine levels by all three assay methods: Emit, RIA, and HPLC.

The Emit® 2000 Cyclosporine Specific Assay results were much higher during the second and third post-transplantation period for all organ type patients experiencing nephrotoxic episodes. During the first month of post-transplantation, the trend of higher cyclosporine levels corresponding with nephrotoxic episodes was less apparent.

A more consistent pattern was observed between cyclosporine levels measured by the Emit® 2000 Cyclosporine Specific Assay and episodes of rejection and nephrotoxicity. For two out of three organ types (heart and kidney), cyclosporine levels tended to be lower with patients with rejection episodes than with patients showing no rejection. No pattern could be drawn from liver patients, possibly due to an insufficient amount of data to make a determination.

Over the course of the clinical trial, the stabilities of the standard calibration curves were reviewed at three study sites. At two of the study sites, no standard curve recalibrations were required during the clinical trial period. At the remaining site, 8 recalibrations were required for the total of 57 runs performed (The mean time between recalibrations was approximately 10 days.). The difference between site performances was based on the QC control ranges generated and used at each trial site. The site which required the 8 standard curve recalibrations, used "tighter" QC control ranges than the other two study sites which did not require any standard curve recalibrations during the study period. Despite the differences in standard curve recalibrations frequencies, the overall performance of the Emit® 2000 Cyclosporine Specific Assay was consistent at all clinical trial sites.

Expected Values

A clinical evaluation of 136 transplant patients (30 heart, 64 kidney, 42 liver) was conducted with the Emit® 2000 Cyclosporine Specific Assay at four study sites. At each site, trough cyclosporine blood levels were serially monitored. Over 4,000 blood samples were analyzed. The patients were followed for an average of 127 days (4.2 months), with a range of 37 to 245 days. Treatment regimens varied among institutions and among patients.

Table 19 shows Emit® 2000 Cyclosporine Specific Assay results over time for 26 transplant patients (9 heart, 6 kidney, 11 liver) who did not experience either moderate or severe rejection, nephrotoxicity, or serum creatinine levels that exceeded 2.0 mg/dL. Although the upper limit of normal for serum creatinine is approximately 1.7 mg/dL, 2.0 mg/dL was selected to allow for the fact that transplant patients have elevated serum creatinine due to a combination of factors including the surgical procedure and the effects of various nephrotoxic drugs.

The information in Table 19 was derived from one cyclosporine measurement in each time period for each patient tested during that time period. Not all patients were tested during each time period.

Table 19
Cyclosporine Levels Over Time in Transplant Patients
Who Did Not Experience Rejection, Nephrotoxicity, or Elevated Serum Creatinine Levels

Time Period	Week 1	Week 2	Week 3	Week 4	Month 2	Month 3	Month 4
Heart Patients							
N	9	9	8	9	9	8	8
Mean (ng/mL)	279	402	527	386	337	343	315
SD (ng/mL)	89	141	187	122	148	226	127
Kidney Patients							
N	5	6	6	6	6	6	5
Mean (ng/mL)	123	240	359	289	270	205	200
SD (ng/mL)	47	132	149	180	122	77	56
Liver Patients							
N	9	10	11	11	10	11	10
Mean (ng/mL)	330	254	288	250	230	276	224
SD (ng/mL)	126	117	46	111	68	104	83

Table 20 shows the distribution of all Emit® 2000 Cyclosporine Specific Assay measurements for the same 26 patients (several measurements are included for each patient).

Table 20
Distribution of Cyclosporine Measurements in Transplant Patients
Who Did Not Experience Rejection, Nephrotoxicity, or Elevated Serum Creatinine

Cyclosporine Concentration	Heart		Kidney		Liver	
	N (Samples)	%	N (Samples)	%	N (Samples)	%
< 50 ng/mL	6	1.9%	1	0.6%	9	2.5%
50 - 100 ng/mL	16	5.1%	10	6.4%	5	1.4%
100 - 150 ng/mL	17	5.4%	26	16.7%	39	10.9%
150 - 200 ng/mL	17	5.4%	34	21.8%	64	17.9%
200 - 250 ng/mL	32	10.2%	26	16.7%	70	19.6%
250 - 300 ng/mL	37	11.8%	24	15.4%	52	14.5%
300 - 350 ng/mL	36	11.5%	16	10.3%	48	13.4%
350 - 400 ng/mL	33	10.5%	5	3.2%	31	8.7%
400 - 450 ng/mL	40	12.7%	3	1.9%	17	4.7%
450 - 500 ng/mL	26	8.3%	5	3.2%	9	2.5%
500 - 550 ng/mL	10	3.2%	3	1.9%	2	0.6%
550 - 600 ng/mL	10	3.2%	0	0.0%	0	0.0%
> 600 ng/mL	34	10.8%	3	1.9%	12	3.4%

By visual inspection of the data presented in Table 20, cyclosporine concentration ranges were selected that were representative of the majority of assay results not associated with rejection, nephrotoxicity, or elevated serum creatinine levels. For kidney patients, 81 per cent of the determinations fell between 100 and 350 ng/mL. Similarly, for liver patients, 76 per cent of the determinations fell between 100 and 350 ng/mL. No attempt was made to identify representative ranges for the nine heart patients because five of these patients, all treated at the same medical center, had significantly higher blood CsA levels than the four remaining patients who were treated at other medical centers.

Based on these observations, cyclosporine concentration ranges for heart, kidney, and liver transplant patients were selected to correlate with the incidence of rejection and nephrotoxicity. (For the purposes of this analysis, the heart cyclosporine concentration range which represented the majority of assay results not associated with nephrotoxicity, rejection, or elevated serum creatinine levels was selected as between 200 to 500 ng/mL).

Cyclosporine concentrations obtained within one week prior to an initial adverse event (i.e., nephrotoxicity or rejection) were first selected for each patient. The number of samples per patient ranged from one to seven. For the purposes of associating the adverse event with the previously described ranges of cyclosporine concentrations by organ, the cyclosporine concentration obtained most immediately prior to the adverse event, was used to construct Table 21, which presents the numbers and percentages of patients with samples in the described cyclosporine concentration ranges who experienced either rejection or nephrotoxicity

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For patients who did not experience rejection and patients who did not experience nephrotoxicity, one sample per week for the first month and one per month for the next two months were included in the calculation.

Table 21
Percentages of Samples in the Described Cyclosporine Concentration Ranges
that were Associated with Either Rejection or Nephrotoxicity

Cyclosporine Concentration	Total Number of Patients	Number of Patients with Rejection/ Nephrotoxicity	Percent of Patients with Rejection/ Nephrotoxicity	95% Confidence Interval
Rejection:				
Heart				
< 200 ng/mL	13	4	30.8%	5.7% to 55.9%
≥ 200 ng/mL	87	8	9.2%	3.1% to 15.3%
Kidney and Liver				
< 100 ng/mL	20	2	10.0%	-3.1% to 23.1%
≥ 100 ng/mL	314	20	4.6%	3.7% to 9.1%
Nephrotoxicity:				
Heart				
< 500 ng/mL	115	4	3.5%	0.1% to 6.8%
≥ 500 ng/mL	28	3	10.7%	-0.7% to 22.2%
Kidney				
< 350 ng/mL	264	7	2.7%	0.7% to 4.6%
≥ 350 ng/mL	21	1	4.8%	-4.3% to 13.9%
Liver				
< 350 ng/mL	126	4	3.2%	0.1% to 6.2%
≥ 350 ng/mL	35	4	11.4%	0.9% to 22.0%

Although the trends indicated in Table 21 are consistent with expected outcomes (i.e., an increased incidence of rejection with low cyclosporine levels and an increased incidence of nephrotoxicity with high cyclosporine levels), no universally applicable conclusions can be drawn because of the small sample size. These results were also observed in previous cyclosporine devices for which there are approved PMAs.

Note: The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of cyclosporine, coadministration 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. Individual cyclosporine values cannot be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment

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adjustments are made, and each user must establish his or her ranges based on clinical experience.

Summary

The nonclinical studies demonstrated that the Emit® 2000 Cyclosporine Specific Assay provided acceptable performance in the areas of precision, cyclosporine recovery, assay sensitivity, cross-reactivity, carryover, dilution parallelism, and susceptibility to potentially interfering substances. Studies also demonstrated the equivalence and acceptable performance of two recommended dilution protocols for patient samples with cyclosporine levels above 500 ng/mL. The studies also showed that the Emit® 2000 Cyclosporine Specific Assay demonstrated acceptable within-run and total precision.

The clinical studies demonstrated that the Emit® 2000 Cyclosporine Specific Assay had acceptable correlation with each of the clinical trial site's established Reference method and HPLC procedures as well as consistency among organ types. In addition, the Emit® 2000 Cyclosporine Specific Assay provided cyclosporine values which were consistent with expected outcomes (i.e., an increased incidence of rejection with low cyclosporine levels and an increased incidence of nephrotoxicity with high cyclosporine levels).

The data presented from the nonclinical and clinical studies demonstrate that the Emit® 2000 Cyclosporine Specific Assay is safe and effective for its intended use when used in accordance with the instructions provided.

The Expected Values section of the package insert for Emit® Cyclosporine Specific Assay, Table 6 and its preceding paragraph, should provide adequate information to the user regarding the described cyclosporine concentration ranges that were associated with either rejection or toxicity. This device does not represent new technology. The EMIT method is the basis for all Behring Diagnostics drug assays.

IX. Panel Recommendation

The Clinical Chemistry and Toxicology Devices Panel recommended at the panel meeting on November 16, 1992 that the PMA for the Emit® 2000 Cyclosporine Specific Assay be approved with conditions and recommended the following revisions to the package insert:

X. CDRH Action on the Application

In June 1993 the sponsor requested a delay in the preapproval inspection until further notice. In September 1995 the applicant submitted documentation indicating a change of ownership from Syva to Behring Diagnostics, Inc., as well as manufacturing process changes, improved quality control, stability, documentation of manufacturing processes, and a redesigned cassette. On November 21, 1995 an amendment was submitted documenting new studies to validate that the manufacturing improvements did not alter the assay which the Clinical Chemistry and Toxicology Devices Panel had

recommended approval at their November 1992 meeting. In March 1996 cited deficiencies were satisfactorily addressed by the sponsor. From April to August of 1996 the FDA's San Francisco District Office had ongoing concerns regarding process validation. These issues were resolved based on the completed device performance studies.

CDRH issued an approval order for the applicant's PMA for the Emit® 2000 Cyclosporine Specific Assay on October 2, 1996.

The applicant's manufacturing and control facilities were inspected on April 12, 1996 and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMPs). The shelf-life of the Emit® 2000 Cyclosporine Specific Assay has been established at 9 months when stored at 2-8° C.

XI. Approval Specifications

Directions for use: See attached labeling

Conditions of Approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order

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5. P.A. Keowan and C.R. Stiller, Cyclosporine: A Double-Edged Sword, Hospital Practice (May 15, 1987) page 207-220.
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10. NCCLS Proposed Guideline EP6-P. Evaluation of the Linearity of Quantitative Analytical Methods. Villanova, PA. National Committee for Clinical Laboratory Standards, 1986.
11. NCCLS Proposed Guideline EP7-P. Interference Testing in Clinical Chemistry. Villanova, PA. National Committee for Clinical Laboratory Standards, 1986.

Emit[®] 2000 Cyclosporine Specific Assay

Caution: United States federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory. Use is restricted to, by, or on the order of a physician.

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Intended Use

The Emit[®] 2000 Cyclosporine Specific Assay is for in vitro diagnostic use on the COBAS MIRA[®], COBAS MIRA S[®], and COBAS MIRA[®] Plus chemistry systems for the quantitative analysis of cyclosporine (CsA) in human whole blood as an aid in the management of cyclosporine therapy in kidney, heart, and liver transplant patients.

CONTRAINDICATIONS:

There are no known contraindications for the Emit[®] 2000 Cyclosporine Specific Assay.

WARNINGS:

1. HANDLE ALL SPECIMENS AS IF CAPABLE OF TRANSMITTING INFECTION.
2. Do not eat, drink, smoke or apply cosmetics where kit reagents are being handled.
3. Do not pipette by mouth.
4. Wear disposable gloves and appropriate laboratory protective apparel while handling samples and kit reagents. Thoroughly wash hands afterwards.
5. Always handle human derived materials as if they were potentially infectious. The calibrators and controls in this kit contain human blood components. Each lot is tested and found to be negative for antibody to human immunodeficiency virus (HIV) and Hepatitis B Surface Antigen (HbsAG).
6. Methanol, which is a material required but not provided for the Emit[®] 2000 Cyclosporine Specific Assay and is used to extract cyclosporine from samples, is flammable. Do not ingest. Avoid skin contact.
7. Assay components contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. After disposing of solutions containing sodium azide in laboratory sinks, flush the drains with a large volume of water to prevent azide buildup (1).
8. This kit contains streptomycin sulfate. Please dispose of appropriately.

PRECAUTIONS:

1. For In Vitro Diagnostic Use only.
2. Do not use the kit after the expiration date.
3. For the disposal of any remaining kit reagents or specimens refer to local regulations for the proper disposal of medical waste.

COBAS MIRA, COBAS MIRA S, and COBAS MIRA Plus are trademarks of Roche Diagnostic Systems, Inc.



Cyclosporine is a cyclic undecapeptide of fungal origin and a potent immunosuppressive agent. Since its introduction in 1983, cyclosporine has substantially improved patient and graft survival in patients receiving heart, kidney, liver, pancreas, or lung transplants. Many studies have documented the effect of cyclosporine in combating organ rejection.

Inadequate cyclosporine doses and levels may result in rejection of the transplanted organ. Toxic levels of cyclosporine are associated with many serious side effects, including nephrotoxicity, hepatotoxicity, and a range of other complications. Physicians are particularly concerned with the nephrotoxic effects of the drug when used in renal transplantation because of the difficulty in distinguishing between organ rejection and cyclosporine toxicity (2,3).

Monitoring parent drug cyclosporine concentrations in whole blood and interpreting these concentrations in conjunction with other laboratory data and clinical considerations is the most effective means of ensuring adequate immunosuppressant therapy for recipients of solid-organ transplants.

Whole blood, rather than plasma, is the matrix of choice for the measurement of cyclosporine since the drug is rapidly distributed into the red blood cells. Because the contribution of the more than 30 cyclosporine metabolites to immunosuppression or toxicity remains uncertain, cyclosporine concentrations should be measured using a method that is specific for the parent drug.

The methods historically used to monitor cyclosporine concentrations in blood include high-performance liquid chromatography (HPLC), radioimmunoassay (RIA), and fluorescence polarization immunoassay (FPIA).

The Emit® 2000 Cyclosporine Specific Assay employs a homogeneous enzyme immunoassay technique used for the analysis of cyclosporine in whole blood. The assay contains mouse monoclonal antibodies with a high specificity for cyclosporine.

The Emit® 2000 Cyclosporine Specific Assay is based on competition for cyclosporine antibody binding sites. Cyclosporine in the sample competes with cyclosporine in Enzyme Reagent B that is labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH). Active (unbound) enzyme converts the oxidized nicotinamide adenine dinucleotide (NAD) in Antibody Reagent A to NADH, resulting in a kinetic absorbance change that can be measured spectrophotometrically. Enzyme activity decreases upon binding to the antibody, allowing the cyclosporine concentration in the sample to be measured in terms of enzyme activity. Endogenous serum G6P-DH does not interfere because the coenzyme NAD functions only with the bacterial (*Leuconostoc mesenteroides*) enzyme employed in the assay.

Before testing with the Emit® 2000 Cyclosporine Specific Assay, samples, calibrators, and controls are pretreated with methanol. Methanol lyses the cells, solubilizes the cyclosporine, and precipitates most of the blood proteins. The samples are centrifuged, and an aliquot of the resulting supernatant containing cyclosporine is diluted with Emit® 2000 Cyclosporine Specific Diluent. This solution is then assayed using Reagents A and B on the COBAS MIRA®, COBAS MIRA S®, or COBAS MIRA® Plus chemistry system.

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, coadministration of other immunosuppressants, type of transplant, time post transplant, and a number of other factors contribute to different requirements for optimal blood levels of cyclosporine. Individual cyclosporine values cannot be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made, and each assay user must establish his or her ranges based on clinical experience. These ranges will vary according to the commercial in vitro diagnostic test used. Ranges must be established for each commercial test used.

(See Section 10, Expected Values)

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Reagents

Catalog Number	Product Description	Quantity/ Volume
6R019UL	Emit® 2000 Cyclosporine Specific Assay	
	Antibody Reagent A* mouse monoclonal antibodies reactive to cyclosporine, nicotinamide adenine dinucleotide, glucose-6-phosphate, sodium chloride, bulking agent, surfactant, and preservatives including 0.1% sodium azide and 0.005% streptomycin sulfate	16.0 mL
	Enzyme Reagent B* cyclosporine labeled with bacterial (<i>Leuconostoc mesenteroides</i>) glucose-6-phosphate dehydrogenase, tris buffer, bulking agents, stabilizers, and preservatives including 0.1% sodium azide and 0.005% streptomycin sulfate	8.0 mL
	Emit® 2000 Cyclosporine Specific Diluent* tris buffer, surfactant, and preservatives including 0.1% sodium azide and 0.005% streptomycin sulfate	100.0 mL
6R119UL	Emit® 2000 Cyclosporine Specific Calibrators** (See below for concentrations) cyclosporine, human whole blood, and preservatives including 0.1% sodium azide	one 2.5 mL vial [†] five 2.0 mL vials

**Reagents and diluent are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.*

***Required for use with the Emit® 2000 Cyclosporine Specific Assay. Supplied separately.*

† Additional negative calibrator is provided as an option for diluting high-concentration samples (see Section 7, Procedure, Diluting High-Concentration Samples).

The calibrators contain the following cyclosporine concentrations:

Calibrator	0	50	100	200	350	500
Cyclosporine (ng/mL)	0	50.0	100.0	200.0	350.0	500.0
Cyclosporine (nmol/L)	0	41.6	83.3	166.5	291.4	416.3

Emit® 2000 Cyclosporine Specific Calibrators are prepared in preserved whole-blood hemolysate. The cyclosporine stock solution for these calibrators is prepared using standard gravimetric procedure, and the concentration of the stock solution is established with high-performance liquid chromatography (HPLC). Aliquots of the stock solution are added to measured amounts of calibrator matrix to yield the desired final concentrations.

Preparation, Storage, and Stability of Assay Components

The Emit® 2000 Cyclosporine Specific Assay reagents and Emit® 2000 Cyclosporine Specific Diluent are provided ready for use. Close the reagent and diluent vials when not in use. Always return the reagent screw caps to their original reagent cassette openings.

Do not freeze reagents and diluent or expose them to temperatures above 27°C. Unopened reagents will remain stable until the expiration date printed on the label if stored at a temperature of 2-8°C. After opening, reagents will remain stable for 12 weeks or until the expiration date printed on the label, whichever comes first, if stored at 2-8°C, upright, and with caps tightly closed.

Note: If reagents turn a deep yellow color, they have deteriorated and should be discarded.

Diluent may be stored either refrigerated or at a room temperature of 18-25°C. However, once the kit is opened, diluent should be stored and used at room temperature. After opening, diluent will remain stable for 6 months or until the expiration date printed on the label, whichever comes first, if stored tightly closed.

Unopened calibrators should be stored frozen at -10°C or below. Prior to initial use, calibrators must be completely thawed with no ice crystals remaining. Allow calibrators to thaw at a room temperature of 18-25°C for approximately one hour or at a refrigerated temperature of 2-8°C overnight. Before use, gently invert the calibrator vials at least ten times to ensure that the contents are thoroughly mixed. **Do not vortex.** Once the calibrators have been thawed, they should be stored at 2-8°C and should not be

refrozen. Calibrators may be used for up to 8 weeks or until the expiration date printed on the label, whichever comes first, when stored at 2-8°C.

Reagents, diluent, and calibrators must be allowed to stand at room temperature (18-25°C) before use. Table 1 lists the minimum times required for the reagents, diluent, and calibrators to stand and also summarizes storage and stability information.

Table 1 -- Storage and Stability of Assay Components

Component	Storage Opened Temp	Minimum Time at 18-25°C Before Use	Stability*	
			Vial	Vial
Reagent A (black cap) wk	2-8°C	60 min**	Exp date	12
Reagent B (white cap) wk	2-8°C	60 min**	Exp date	12
Diluent	Unopened kit: 2-8°C	2 hr	Exp date	N/A
	Opened kit: 18-25°C	N/A	Exp date	6 mo
Calibrators	Unopened: < -10°C	1 hr	Exp date	N/A
	Opened: 2-8°C	30 min	N/A	8 wk

**Stability depends on handling components as directed.*

***If using a refrigerated compartment on the COBAS MIRA S® or COBAS MIRA® Plus system, reagents can be used directly from the refrigerator.*

The Emit[®] 2000 Cyclosporine Specific Assay is performed on the COBAS MIRA[®], COBAS MIRA S[®], and COBAS MIRA[®] Plus chemistry systems. All of these systems have a level detector on the reagent probe that senses when the volume of Reagent A is too low to run the assay. The COBAS MIRA[®] Plus chemistry system also has a level detector for Reagent B. However, on the COBAS MIRA[®] and COBAS MIRA S[®] systems, Reagent B is dispensed by the sample needle, which does not have a level detector. **Therefore, the operator must confirm that there is sufficient Reagent B to run the assay.**

Refer to instrument manuals for programming the COBAS MIRA[®], the COBAS MIRA S[®], and the COBAS MIRA[®] Plus chemistry systems.

-
- The required sample volume is 100 μ L of whole blood.
 - Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. These factors include dosage, mode of administration, concomitant drug therapy, and biological variations affecting drug disposition. A trough sample is recommended for measurement of cyclosporine.
 - Blood should be drawn using tubes containing the anticoagulant EDTA. EDTA is recommended as the anticoagulant of choice for assaying cyclosporine in whole-blood samples. Heparinized samples are not recommended because they may form clots during storage.
 - Use fresh samples. If samples are to be tested within 8 hours of collection, they may be stored at a room temperature of 18-25°C. They may be stored refrigerated at 2-8°C for up to one week. If longer storage is necessary, samples should be frozen at -20°C. Cyclosporine has been shown to be stable in whole-blood samples for at least 3 months when stored at -20°C (4,5). Thaw and thoroughly mix frozen samples before testing. Repeated freeze-thaw cycles should be avoided. Insoluble materials that may form when some samples are frozen should be avoided when pipetting.
 - All specimens should be handled as if capable of transmitting disease. Follow standard precautions for handling infectious agents during all procedures (6,7):

Materials Required***Provided by Behring Diagnostics Inc.***Emit[®] 2000 Cyclosporine Specific Assay

Antibody Reagent A and Enzyme Reagent B

Emit[®] 2000 Cyclosporine Specific DiluentEmit[®] 2000 Cyclosporine Specific Calibrators***Available from Behring Diagnostics Inc.***COBAS MIRA[®], COBAS MIRA S[®], or COBAS MIRA[®] Plus chemistry system***Available from Roche Diagnostic Systems***Reagent 5_S racks

Cal CS 30 calibrator racks

Sample rack (Sample 30) and Rack cover

COBAS MIRA[®], COBAS MIRA S[®], or COBAS MIRA[®] Plus cuvette segments

Sample cups

Materials Required But Not Provided

Multilevel whole-blood controls

200 µL positive displacement pipette or Eppendorf Repeater (for delivery of methanol)

100 µL or 200 µL positive displacement pipette (for dispensing samples)

Pipetter-diluter with 100 µL sample pickup and 200 µL diluent dispense (for transfer of extract to COBAS MIRA[®] sample cup; positive displacement pipette also may be used)*

Eppendorf Combitips (5.0 mL) if Eppendorf Repeater is used

Microcentrifuge tubes (1.5 to 2.0 mL capacity)

Distilled or deionized water in plastic wash bottle

Methanol (reagent grade or better)

Microcentrifuge

Microcentrifuge tube rack

Vortex mixer

Laboratory tissues

Sample inverter or rocker (optional)

****Suggested specifications for pipetter-diluter (based on gravimetric testing): accuracy ±3% sample syringe, ±2% diluent syringe; precision 1% CV sample and diluent syringe.***

General Instrument Usage Instructions

1. If necessary, set the instrument to 37°C.
 - To program the temperature, press <PROGRAM>, <6> SYSTEM PARAMETERS, and <1> ANALYTICAL PARAMETERS.
2. To program the assay, press <PROGRAM>, <2> TESTS. The screen will display the Assignment Table.
 - Select an undefined letter key.
 - Type the TEST NAME (up to four characters).
 - Press <ENTER>. The cursor/highlighter will advance to COPY FROM.
 - Press <ENTER> to bypass this option.
 - The first page of the test parameters with the default values will appear.
 - Proceed to enter the instrument settings for either the COBAS MIRA[®], COBAS MIRA S[®], or COBAS MIRA[®] Plus chemistry system (given below). Press <ENTER> or <↓> to advance to the next parameter.

Setup Procedure

1. Allow all reagents, samples, calibrators, and controls to stand at room temperature (18-25°C) before use (see Table 1 for minimum times). If using a refrigerated compartment on the COBAS MIRA S[®] or COBAS MIRA[®] Plus system, reagents can be used directly from the refrigerator. However, samples and all other assay components must be allowed to stand at room temperature before use.
2. Equilibrate and use both diluent and methanol at room temperature (18-25°C). Note that both may be stored at room temperature.
3. Prepare, store, and use controls according to manufacturer's directions.
4. Turn on the COBAS MIRA[®] system. Ensure that the system is programmed according to the appropriate protocol in Section 13, Appendix. Refer to the appropriate COBAS MIRA[®] system operator's manual for complete instructions.
5. If using an automatic pipetter diluter, check settings and prime the pipetter-diluter with Emit[®] 2000 Cyclosporine Specific Diluent. Ensure that there are no air bubbles in the lines.
6. Set up and label one microcentrifuge tube for each calibrator, control, and sample to be pretreated.
7. Set up the same number of COBAS MIRA[®] sample cups on the COBAS MIRA[®] calibrator and/or sample rack(s).

Pretreatment and Assay Procedure

The procedure has been separated into two sections, Steps and Technical Notes, for easy reference. The technical notes are an essential part of the instructions and must be read thoroughly before completing each step of the procedure.

Note: To minimize carryover, properly maintain your instrument and sample handling equipment according to the manufacturers' instructions, and carefully follow the assay procedure as outlined in both the steps and the technical notes. See Section 8, Evaluation of Results, for further information.

The reproducibility and accuracy of the sample and methanol pipetting devices are crucial to the success of the method. Periodic calibration must be performed. Proper use of these devices is also essential.

STEPS	TECHNICAL NOTES
<p>1. Mix all calibrators, controls, and samples gently but thoroughly just before use.</p>	<ul style="list-style-type: none"> • Do not vortex. The liquids may be mixed by hand or on an inverter or rocker. • The calibrators are a whole-blood hemolysate and may be slightly different in appearance from whole-blood samples.
<p>2. Transfer 100 µL (see first technical note for step 2) of each calibrator, control, and/or sample to the appropriately labeled microcentrifuge tube using a positive displacement pipette.</p>	<ul style="list-style-type: none"> • Alternatively, 200 µL of sample may be extracted with 400 µL of methanol. These larger volumes make it easier to avoid the precipitate when removing aliquots of sample supernatant with the pipetter-diluter (see step 7). • A single capillary tube may be used to dispense all of the samples, calibrators, and controls provided that the outside of the capillary barrel and the plunger tip are thoroughly wiped between samples with a moist laboratory tissue.
<p>3. Add 200 µL (see first technical note for step 2) of methanol to each microcentrifuge tube with a positive displacement pipette. Immediately cap each tube.</p>	<ul style="list-style-type: none"> • A positive displacement pipette or Eppendorf Repeater must be used for dispensing methanol because the viscosity of methanol is low relative to that of water.

<p>4. Vortex each microcentrifuge tube for at least ten seconds.</p>	<ul style="list-style-type: none"> • Vortexing soon after adding the methanol will minimize the time needed to break up any pellets that may form. Sample plus methanol mixture should be completely homogeneous immediately after vortexing.
<p>5. Incubate the contents of the microcentrifuge tubes at room temperature (18-25°C) for at least one minute after vortexing of the last sample is completed.</p>	<ul style="list-style-type: none"> • Microcentrifuge tubes may incubate up to one hour after vortexing and before centrifuging.
<p>6. Spin the microcentrifuge tubes in a microcentrifuge for at least two minutes.</p>	<ul style="list-style-type: none"> • The supernatant may appear slightly yellow or green, but it should not be cloudy. If the supernatant is cloudy or becomes cloudy on standing, it should be recentrifuged (g force x minutes³ 25,000 g-min).
<p>7. Carefully pick up 100 µL of supernatant with the pipetter-diluter or a positive displacement pipette. Dispense the supernatant with 200 µL of Emit® 2000 Cyclosporine Specific Diluent directly into the COBAS MIRA® sample cup. Immediately cap sample cup after each sample is dispensed.</p>	<ul style="list-style-type: none"> • Avoid the precipitate when removing aliquots of sample supernatant with the pipetter-diluter. • The inside diameter of the tubing on the pipetter-diluter should be less than 1 mm to minimize sample carryover. • The pipetter-diluter tip should be wiped between samples to minimize carryover.

<p>8. Mix the diluted supernatants by covering the sample and/or calibration rack(s) with the rack cover and completely inverting the rack(s) at least ten times. Then gently tap the rack(s) to release any air bubbles that are trapped at the bottom of the cups.</p>	<ul style="list-style-type: none"> • To guard against obtaining an assay value when no sample is present, ensure that all sample cups contain sample. • The diluted supernatants may remain in unpierced sample cups not longer than 4 hours before being analyzed. Once pierced, diluted supernatants may be reused for an additional 2 hours, provided total time since preparation does not exceed 4 hours. • The volume of each diluted supernatant is sufficient for six replicate assays.
<p>9. Place racks onto the rack platform.</p>	<p>(none)</p>
<p>10. Mix reagents gently but thoroughly and place into appropriate positions on the Reagent 5_s rack.</p>	<ul style="list-style-type: none"> • Do not vortex. Reagents may be mixed by hand or on an inverter or rocker. If a bubble covers the mouth of either reagent vial, pierce the bubble with a clean implement.
<p>11. Insert empty cuvette segments into position.</p>	<p>(none)</p>
<p>12. Prime syringes by pressing <INFO> and <6> SYSTEM CHECKS keys. Press <1> PRIME. The screen will respond with "PRIME". Press <F1> START to prime. Allow the system to prime five complete cycles. Press <F1> again to stop priming.</p>	<ul style="list-style-type: none"> • Check the syringes for bubbles. Check the liquid streams exiting the probes for steady flow. If necessary, remove bubbles from syringes and/or replace probe(s).

<p>13. <i>To Precalibrate:</i> Press <ROUTINE>, then in place of sample position number, type "PC". The screen will respond with "PCAL". Select the tests to be precalibrated, then press <ENTER>. Proceed to step 16.</p> <p><i>To Calibrate:</i> Press <ROUTINE>, then in place of sample position number, type "CA". The screen will respond with "CAL". Select the tests to be calibrated, then press <ENTER>. Proceed to step 14.</p>	<ul style="list-style-type: none"> • Precalibrate when running calibrators alone (no samples or controls). • Calibrate when running samples and/or controls with calibrators.
<p>14. <i>To Program the Worklist:</i> Press <ROUTINE>. Type in the sample position(s) and the appropriate Test Keys (tests to be performed) for each, followed by pressing <ENTER>. If duplicates are desired, press appropriate Test Keys twice, and then press <ENTER>.</p>	<ul style="list-style-type: none"> • Ensure that the programmed routine corresponds to the placement of sample cups within the sample rack.
<p>15. To run controls or patient samples from a stored curve, program the <ROUTINE> worklist as in step 14, press <ENTER> and then press <START>.</p>	<p>(none)</p>

<p>16. Press <STATUS>. The STATUS screen will display "RACK HANDLING POSSIBLE" and "SEGMENT HANDLING POSSIBLE". Press <START>. If the analysis does not start, the instrument will display a system message at the bottom of the CRT. Refer to the operator's manual for more information.</p>	<p>(none)</p>
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Calibration

1. Calibrate whenever a new lot of reagents is used. Recalibration may be necessary when significant changes to the instrument (eg, changes to the pipetting and photometry systems) are made during instrument maintenance.
2. Verify that the chemistry system is operating correctly by following instructions in the instrument operator's manual.
3. Pretreat a set of Emit[®] 2000 Cyclosporine Specific Calibrators according to the sample pretreatment protocol. Calibrators may be pretreated along with samples and controls.
4. Place the pretreated calibrators in the appropriate COBAS MIRA[®] calibrator rack and calibrate according to instructions in the instrument operator's manual.
5. Accept the calibration if each bi-level or tri-level control falls within control limits (see below for information on establishing control limits).

Quality Control

Temporary Control Limits

1. After initial calibration, assay three replicates each of multi-level (2 or more) controls in a single run. Testing controls in the order of high to low is recommended. Record control results. **Do not** discard any control result unless it was generated by operator error or instrument malfunction or unless the control result can be rejected by a statistical outlier test.
2. Repeat calibration, assaying of controls, and recording of control results as described in step 1 two more times. Nine results at each level must be recorded from the three runs.
3. Calculate a mean control concentration for each control level based on the nine determinations generated for that level.
4. Define *temporary* control limits for each control level using the mean control concentrations determined in step 3 and referring to Table 2. For example, if the mean control concentration is 100 ng/mL, then the control limits will be 70 ng/mL (-30%) and 130 ng/mL (+30%).

Table 2 -- Control Limits

Control*	Mean Control Concentration (ng/mL)	Limit
Low**	50-100	mean \pm 30%
	101-150	mean \pm 20%
Medium	151-300	mean \pm 20%
High	301-400	mean \pm 20%

**Use bi-level (low and high) or tri-level controls.*

***Run only one level of low control. If the low control falls within 50-100 ng/mL, set control limits at mean \pm 30%. If the low control falls within 101-150 ng/mL, set control limits at mean \pm 20%.*

5. Use the established *temporary* limits for at least 30 calendar days when running patient samples. In order to establish *permanent* limits, collect a minimum of 20 determinations at each control level and recalibrate at least every 10 days during the period in which the *temporary* limits are used.

Note: The temporary control limits should be appropriate throughout the 30 calendar days. However, if any control exhibits a consistent testing bias relative to its temporary limits, re-establish the temporary control limits by repeating steps 1 through 5, above. If difficulty in using the temporary control limits continues, call a technical consultant for assistance.

Permanent Control Limits

1. Collect control results during the 30 calendar days (a minimum of 20 determinations at each level) that *temporary* control limits are being used. Testing controls in the order of high to low is recommended. Do not discard any control result unless it was generated by operator error or instrument malfunction or unless the control result can be rejected by a statistical outlier test.
2. Recalculate the mean and standard deviation of the control concentrations at each level, including all results collected in step 1 plus the nine results obtained when establishing *temporary* limits.
3. *Permanent* control limits should be set at ± 2.25 SD of the mean, provided these limits are not less than $\pm 12\%$ of the mean and not greater than $\pm 25\%$ of the mean. If ± 2.25 SD of the mean is less than $\pm 12\%$ of the mean, set the *permanent* control limits at $\pm 12\%$ of the mean. If ± 2.25 SD of the mean is greater than $\pm 25\%$ of the mean, set the *permanent* control limits at $\pm 25\%$ of the mean. Once at least 20 calibration curves have been obtained, the *permanent* control limits may be re-established by calculating the mean and multiplying the standard deviation for each control level by 2.
4. Establish new permanent control limits whenever a new lot of controls is used. New limits can be established by testing the new controls in 20 runs that are verified using the former controls.

Daily Quality Control

1. Assay each control level for the bi-level or tri-level controls at least once in every run. Testing controls in the order of high to low is recommended.
2. Verify each run using the following instructions:
 - If controls are within their control limits, accept the run.
 - If any control is not within its control limits, examine all materials, check for operator error or instrument malfunction, and then rerun that control. If, after retesting, the control is within its control limits, accept the run.
 - If the control is still not within its control limits, recalibrate. If, after recalibration, controls are within their control limits, accept the run.
 - If, after recalibration, any control is not within its control limits, examine all materials, check for operator error or instrument malfunction, and then rerun that control. If, after retesting, the control is within its control limits, accept the run.
 - If, after retesting, the control is still not within its control limits, call a technical consultant for assistance.

Note: If more frequent verification of test results is required by the operating procedures within your laboratory, those requirements should be met.

Diluting High-Concentration Samples

If a pretreated patient sample assays higher than 500 ng/mL (416.3 nmol/L) cyclosporine (ie, the printout reads "OUT OF TEST RANGE"), use the following directions to manually dilute an aliquot of assayed patient sample (taken from the COBAS MIRA® sample cup) with a 25% solution of methanol in Emit® 2000 Cyclosporine Specific Diluent.

1. Prepare a 25% solution of methanol in Emit® 2000 Cyclosporine Specific Diluent.
 - a. Pipette 2.5 mL methanol (reagent grade or better) into a 10-mL volumetric flask.
 - b. Bring the liquid volume to 10 mL with Emit® 2000 Cyclosporine Specific Diluent.
 - c. Mix the solution thoroughly by repeated inversion.

Minimize exposure of the solution to air. The solution may be used for up to one week after preparation when stored at room temperature in a tightly sealed container.

2. Dilute and assay pretreated high-concentration samples.
 - a. In a clean COBAS MIRA[®] sample cup, combine one part assayed patient sample (taken from the original COBAS MIRA[®] sample cup) with two parts 25% solution of methanol in Emit[®] 2000 Cyclosporine Specific Diluent (prepared according to directions given above).

The minimum volume needed to run two replicates is 125 µL for the COBAS MIRA[®] and COBAS MIRA S[®] systems and 175 µL for the COBAS MIRA[®] Plus system.
 - b. Immediately cap the diluted sample.
 - c. Assay the diluted sample using steps 8 through 16 of the Pretreatment and Assay Procedure.
 - d. Multiply the assay result by 3 to obtain an estimate of the cyclosporine concentration.

Note: Diluted supernatants may be used for 2 hours following initial assay, provided total time since preparation does not exceed 4 hours.

Alternatively, whole-blood high-concentration samples may be diluted with the negative whole-blood calibrator using the following directions:

1. Mix negative whole-blood calibrator and whole-blood high-concentration samples gently but thoroughly just before use.
2. Combine one part whole-blood sample with two parts negative whole-blood calibrator.
3. Mix the solution thoroughly by repeated inversion.
4. Pretreat and assay the diluted whole-blood sample using steps 1 through 16 of the Pretreatment and Assay Procedure.
5. Multiply the assay result by 3 to obtain an estimate of the cyclosporine concentration.

Results are calculated automatically by the COBAS MIRA[®], COBAS MIRA S[®], or COBAS MIRA[®] Plus system software. Consult the operator's manual for complete instructions.

The effect of carryover should be considered when evaluating a low concentration sample that follows a sample with a cyclosporine concentration of 500 ng/mL or higher. The amount of carryover varies from system to system; studies have indicated that carryover can be up to 4% (eg, 2.0 ng/mL from a 50 ng/mL sample or 32 ng/mL from an 800 ng/mL sample). To minimize carryover, properly maintain your instrument and sample handling equipment according to the manufacturers' instructions and carefully follow the assay procedure as outlined in both the steps and the technical notes.

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- The Emit® 2000 Cyclosporine Specific Assay is for in vitro diagnostic use in the measurement of cyclosporine in whole blood. This assay is not intended to be used for measuring cyclosporine in serum or plasma.
 - One potential source of error is carryover. The amount of carryover varies from system to system; studies have indicated that carryover can be up to 4%.
 - Do not use a bleach solution to flush a COBAS MIRA®, COBAS MIRA S®, or COBAS MIRA® Plus system. Using a bleach solution will negatively affect assay performance.

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, coadministration of other immunosuppressants, type of transplant, time post transplant, and a number of other factors contribute to different requirements for optimal blood levels of cyclosporine. Individual cyclosporine values cannot be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made, and each assay user must establish his or her ranges based on clinical experience. These ranges will vary according to the commercial in vitro diagnostic test used. Ranges must be established for each commercial test used.

A clinical evaluation of the Emit[®] 2000 Cyclosporine Specific Assay was conducted in heart, kidney, and liver transplant patients at three study sites, and at a fourth site in kidney and liver transplant patients. At each site, trough cyclosporine blood levels were serially monitored.

One hundred and thirty-two patients were included in this study: 30 heart transplant patients, 65 kidney transplant patients, and 37 liver transplant patients. Over 4000 blood samples were analyzed. The patients were followed for an average of 127 days (4.1 months), with a range of 37 to 312 days. Treatment regimens varied among institutions and among patients.

Table 3 shows Emit[®] 2000 Cyclosporine Specific Assay results over time for 26 patients (9 heart, 6 kidney, and 11 liver transplant patients) who did not experience rejection defined as moderate or severe nephrotoxicity, or serum creatinine levels that exceeded 2.0 ng/dL. Although the upper limit of normal for serum creatinine is approximately 1.7 ng/dL, 2.0 ng/dL was selected to allow for the fact that transplant patients have elevated serum creatinine due to a combination of factors including the surgical procedure and the effects of various nephrotoxic drugs. The information in Table 3 was derived from one cyclosporine measurement in each time period for each patient tested during that time period. Not all patients were tested during each time period.

Table 3 -- Cyclosporine Levels Over Time in Patients Who Did Not Experience Rejection, Nephrotoxicity, or Elevated Serum Creatinine

Time Period	Kidney (6 patients)			Liver (11 patients)			Heart (9 patients)		
	N (patients)	Mean (ng/mL)	SD (ng/mL)	N (patients)	Mean (ng/mL)	SD (ng/mL)	N (patients)	Mean (ng/mL)	SD (ng/mL)
Week 1	5	123	47	9	330	126	9	279	89
Week 2	6	240	132	10	254	117	9	402	141
Week 3	6	359	149	11	288	46	8	527	187
Week 4	6	289	180	11	250	111	9	386	122
Month 2	6	270	122	10	230	68	9	337	148
Month 3	6	205	77	11	276	104	8	343	226
Month 4	5	200	56	10	224	83	8	315	127

Table 4 shows the distribution of all Emit[®] 2000 Cyclosporine Specific Assay measurements for the same 26 patients; several measurements are included for each patient.

Table 4 -- Distribution of Cyclosporine Measurements in Patients Who Did Not Experience Rejection, Nephrotoxicity, or Elevated Serum Creatinine

Cyclosporine Concentration	Kidney (6 patients)		Liver (11 patients)		Heart (9 patients)	
	N (samples)	%	N (samples)	%	N (samples)	%
< 50 ng/mL	1	0.6%	9	2.5%	6	1.9%
50-100 ng/mL	10	6.4%	5	1.4%	16	5.1%
100-150 ng/mL	26	16.7%	39	10.9%	17	5.4%
150-200 ng/mL	34	21.8%	64	17.9%	17	5.4%
200-250 ng/mL	26	16.7%	70	19.6%	32	10.2%
250-300 ng/mL	24	15.4%	52	14.5%	37	11.8%
300-350 ng/mL	16	10.3%	48	13.4%	36	11.5%
350-400 ng/mL	5	3.2%	31	8.7%	33	10.5%
400-450 ng/mL	3	1.9%	17	4.7%	40	12.7%
450-500 ng/mL	5	3.2%	9	2.5%	26	8.3%
500-550 ng/mL	3	1.9%	2	0.6%	10	3.2%
550-600 ng/mL	0	0.0%	0	0.0%	10	3.2%
> 600 ng/mL	3	1.9%	12	3.4%	34	10.8%

By visual inspection of the data presented in Table 4, ranges were identified that were representative of the majority of assay results not associated with rejection, nephrotoxicity, or elevated serum creatinine. For kidney transplant patients, 81% of the determinations fell between 100 and 350 ng/mL. Similarly, for liver transplant patients, 76% of the determinations fell between 100 and 350 ng/mL. No attempt was made to identify representative ranges for the nine heart patients because five of these patients, all treated at the same medical center, had significantly higher blood cyclosporine levels than the four patients who were treated at other medical centers. No universally applicable conclusions should be drawn from the data presented above.

Representative samples used to calculate the percentages presented in Tables 5 and 6 were selected as follows. For patients who experienced either rejection or nephrotoxicity, samples drawn just prior to (within one week of) the episode were selected. For patients who did not experience rejection and also for patients who did not experience nephrotoxicity, one sample per week for the first month and one per month for the next two months were included in the calculation.

Table 5 -- Numbers and Percentages of Patients with Samples in the Described Cyclosporine Concentration Ranges Who Experienced Rejection

Cyclosporine Concentration	Number of Patients	Number of Patients with Rejection	Percent of Patients with Rejection	95% Confidence Interval
<i>Heart</i>				
< 200 ng/mL	13	4	30.8%	5.7% to 55.9%
≥ 200 ng/mL	87	8	9.2%	3.1% to 15.3%
<i>Kidney and Liver</i>				
< 100 ng/mL	20	2	10.0%	-3.1% to 23.1%
≥ 100 ng/mL	314	20	4.6%	3.7% to 9.1%

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Table 6 -- Numbers and Percentages of Patients with Samples in the Described Cyclosporine Concentration Ranges Who Experienced Nephrotoxicity

Cyclosporine Concentration	Number of Patients	Number of Patients with Nephrotoxicity	Percent of Patients with Nephrotoxicity	95% Confidence Interval
<i>Heart</i>				
< 500 ng/mL	115	4	3.5%	0.1% to 6.8%
≥ 500 ng/mL	28	3	10.7%	-0.7% to 22.2%
<i>Kidney</i>				
< 350 ng/mL	264	7	2.7%	0.7% to 4.6%
≥ 350 ng/mL	21	1	4.8%	-4.3% to 13.9%
<i>Liver</i>				
< 350 ng/mL	126	4	3.2%	0.1% to 6.2%
≥ 350 ng/mL	35	4	11.4%	0.9% to 22.0%

Although the trends indicated in Tables 5 and 6 are consistent with expected outcomes (i.e., an increased incidence of rejection with low cyclosporine levels and an increased incidence of nephrotoxicity with high cyclosporine levels), no universally applicable conclusions can be drawn because of the small sample size.

Performance characteristics of the Emit® 2000 Cyclosporine Specific Assay are affected by all parameters of the measurement. The following information represents total system performance and should not be interpreted to pertain only to reagents and calibrators.

Reportable Range

The Emit® 2000 Cyclosporine Specific Assay quantitates cyclosporine concentrations in human whole blood containing 40-500 ng/mL cyclosporine. Quantitative results up to 1500 ng/mL can be estimated by diluting and reassaying high-concentration samples and then multiplying the results by the dilution factor (see Section 7, Procedure, Diluting High-Concentration Samples).

Sensitivity

The sensitivity of the Emit® 2000 Cyclosporine Specific Assay is 40 ng/mL. This is the lowest concentration of cyclosporine that can be distinguished from 0.0 ng/mL with a confidence level of 95%.

Specificity

Compounds whose chemical structure or concurrent therapeutic use would suggest possible interference have been tested. The compounds listed in Table 7 do not interfere with the Emit® 2000 Cyclosporine Specific Assay when tested in the presence of 200 ng/mL cyclosporine. Levels tested were determined according to the National Committee for Clinical Laboratory Standards (NCCLS) proposed guideline for interference studies (8).

Table 7 -- Co-administered Drugs and Drug Metabolites That Do Not Interfere

Drug	Test Level ($\mu\text{g/mL}$)	Drug	Test Level ($\mu\text{g/mL}$)
acetaminophen	200	ketoconazole	70
albuterol	0.18	lidocaine	60
allopurinol	60	lovastatin	4
alprazolam	0.37	methylprednisolone	12
amitriptyline	20	metoclopramide	4
amphotericin B	20	mycophenolic acid	100
atenolol	40	mycophenolic acid glucuronid	1000
azathioprine	10	misoprostol	0.015
captopril	50	morphine sulfate	6
carbamazepine	120	Muromonab-CD3	1
cefaclor	230	naproxen	1000
chloramphenicol	250	nitroglycerin	5
cimetidine	100	omeprazole	14
ciprofloxacin	43	phenobarbital	150
cyclophosphamide	250	phenytoin	100
digoxin	0.02	piperacillin	8
dipyridamole	25	prazosin	3
disopyramide	30	prednisolone	12
encainide	1050	prednisone	12
erythromycin	200	promethazine	10
ethanol	3500	ranitidine	200
fluconazole	81	rapamycin	0.1
furosemide	20	salicylic acid	500
ganciclovir	400	sulfamethoxazole	400
gentamicin	120	tacrolimus	0.1
heparin	8000 U/L	theophylline	250
hydralazine	32	triamterene	2.8
hydrochlorothiazide	40	trimethoprim	20
immune globulin	5000	valproic acid	500
isoniazid	70	vancomycin	630
isoproterenol hydrochloride	0.06		

Cross-reactivity with four major cyclosporine metabolites was evaluated in the presence of 200 ng/mL cyclosporine. Percent cross-reactivity, reported in Table 8, was determined by subtracting the actual cyclosporine concentration (200 ng/mL) from the apparent cyclosporine concentration, dividing this number by the concentration of added metabolite, and expressing the result as a percentage.

Table 8 -- Metabolite Cross-Reactivity in the Presence of Cyclosporine

Cyclosporine Metabolite	Metabolite Level Tested (ng/mL)	Cross-reactivity (%)
AM1 (M17)	500	<0.3
AM19 (M8)	500	3.0
AM4N (M21)	500	<0.3
AM9 (M1)	670	7.3

Endogenous Substances

No interference has been found in samples to which 40 mg/dL bilirubin, 20 mg/dL uric acid, 3000 mg/dL triglycerides, or 500 mg/dL cholesterol were added.

Drug Interactions

The following information, reported by Sandoz Pharmaceuticals, is taken from the Physicians' Desk Reference (9):

Cyclosporine is extensively metabolized by the liver. Therefore, circulating cyclosporine levels may be influenced by drugs that affect hepatic microsomal enzymes, particularly the cytochrome P-450 system. Substances known to inhibit these enzymes will decrease hepatic metabolism and increase cyclosporine levels. Substances that are inducers of cytochrome

P-450 activity will increase hepatic metabolism and decrease cyclosporine levels.

Monitoring of circulating cyclosporine levels and appropriate Sandimmune® (cyclosporine) dosage adjustment are essential when these drugs are used concomitantly

Drugs That Increase Cyclosporine Levels

diltiazem	ketoconazole
nicardipine	fluconazole
verapamil	itraconazole
danazol	erythromycin
bromocriptine	methylprednisolone
metoclopramide	

Drugs That Decrease Cyclosporine Levels

rifampin	phenytoin
phenobarbital	carbamazepine

Precision

Total precision and its within-run precision component were determined at three trial sites according to the National Committee for Clinical Laboratory Standards (NCCLS) tentative guideline for evaluation of precision (10). BioRad tri-level controls were tested in triplicate, two runs per day for 20 days (N = 120). Total precision was determined nine times, using two separate analyzers (one each of the COBAS MIRA® and COBAS MIRA S® chemistry systems) and three reagent lots (each with a separate calibrator lot). Table 9 shows the average mean value

(n = 9) for each control level, the standard deviation (SD) ranges, and the % coefficient of variation (%CV) ranges obtained.

Table 9 -- Precision

Control Level (ng/mL)*	Within-run Precision		Total Precision	
	SD Range	%CV Range	SD Range	%CV Range
72	4.4 - 8.5	6.0 - 11.8	7.6 - 13.8	10.4 - 19.1
178	6.9 - 11.2	4.0 - 6.2	10.7 - 16.8	6.0 - 10.0
414	14.8 - 26.1	3.8 - 6.1	26.9 - 35.6	6.3 - 8.5

**Average of nine mean values determined for three reagent lots on three analyzers. Separate calibrator lots were used for each reagent lot. The mean ranged from 63.3 to 77.8 ng/mL for control level 1, from 166.7 to 187.4 ng/mL for control level 2, and from 390.6 to 436.1 ng/mL for control level 3.*

Internal precision studies have shown that the precision obtained with the COBAS MIRA® Plus chemistry system is comparable to that of the COBAS MIRA® and COBAS MIRA S® chemistry systems.

Accuracy

Patients' blood cyclosporine levels were monitored starting immediately post-transplant and continuing for approximately three months. Site 1 monitored levels from kidney and liver transplant patients. Sites 2, 3, and 4 monitored levels from kidney, liver, and heart transplant patients. Samples were analyzed by the Emit® 2000 Cyclosporine Specific Assay and by two reference methods: a radioimmunoassay (RIA) employing a specific monoclonal antibody and high-performance liquid chromatography (HPLC) procedures. A comparative analysis of the results is shown in Table 10.

Table 10 -- Comparative Analysis

Site	Slope	Inter.	R	SEE	No. Samples	No. Patients	Xavg	Yavg
RIA vs Emit assay								
Site 1	0.82	24	0.968	26.78	593	21	269.9	245.1
Site 2	0.91	-4	0.934	40.43	1113	37	265.1	236.6
Site 3	0.79	8	0.929	35.69	786	38	309.7	252.5
Site 4	0.84	13	0.981	24.56	1650	40	311.3	275.9
HPLC vs Emit assay								
Site 1	1.02	24	0.965	25.39	595	21	217.5	244.9
Site 2	1.14	-3	0.917	39.71	1113	37	205.7	231.9
Site 3	0.92	9	0.828	50.68	1030	38	277.7	263.1
Site 4	1.05	12	0.975	25.33	1645	40	252.6	277.0
HPLC vs RIA								
Site 1	1.24	0	0.976	23.66	595	21	220.1	272.7
Site 2	1.29	-3	0.912	43.17	1045	37	207.9	265.3
Site 3	1.12	-1	0.818	59.09	663	38	279.5	312.2
Site 4	1.24	-1	0.977	26.38	1648	40	252.6	312.5

The correlation data shown in Table 10 represent the results of studies done in a number of laboratories. The differences in the correlation statistics are typical of the laboratory-to-laboratory differences that may be seen when any one or a combination of the described assays is used. Neither this correlation data nor other correction factors should be used to convert values between methods. Any comparison of methods should be done only after careful standardization of the methods has been achieved.

Recovery

Cyclosporine recovery was assessed using cyclosporine-free whole blood to which parent cyclosporine was added. Whole blood was spiked to 85 ng/mL and 425 ng/mL cyclosporine. Three aliquots of each spike were pretreated, pooled, and assayed ten times. Recovery study results are shown in Table 11.

Table 11 -- Recovery Study

Nominal Value (ng/mL)	85	425
Range of Mean Measured Values (ng/mL)	82.5-90.7	408.4-459.4
Range of Percent Recovery	97%-106.7%	96.1%-108.1%

A separate study was conducted to determine the effect of hematocrit on the recovery of cyclosporine from a sample. Red blood cells were added to plasma to create five whole-blood samples with hematocrits ranging from 15% to 59%. Nominal concentrations of 75 ng/mL and 425 ng/mL cyclosporine were added to separate aliquots of each of the samples. These aliquots were then assayed. As can be seen in Table 12, the influence of hematocrit on cyclosporine recovery was negligible.

Table 12 -- Effect of Hematocrit on Cyclosporine Recovery

Hematocrit (%)	15	26	37	46	59
<i>75 ng/mL Cyclosporine</i>					
Recovery (%)	109	104	111	115	111
%CV	5.0	6.3	4.1	3.3	7.4
<i>425 ng/mL Cyclosporine</i>					
Recovery (%)	91.3	97.7	97.5	101	101
%CV	6.4	5.3	7.5	7.9	8.7

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Linearity

The linearity of the Emit[®] 2000 Cyclosporine Specific Assay was assessed according to the National Committee for Clinical Laboratory Standards (NCCLS) proposed guideline for evaluation of linearity (11). The assay was found to be linear within the range 40 to 500 ng/mL. The information in Table 13 was determined by plotting target value (x) versus analytical result (y).

Table 13 -- Linearity Study

	COBASMIRA[®] Plus Analyzer	COBASMIRA[®] Analyzer	COBASMIRA S[®] Analyzer
N	20	20	20
Slope	0.97	0.98	1.05
Intercept	-4.5	3.9	-13.3
p-value	0.82	0.70	0.72

-
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 6. Biosafety in Microbiological and Biomedical Laboratories, JY Richmond, RW McKinney (eds). Atlanta, GA, US Dept of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention; Bethesda, MD, National Institutes of Health, 1993.
 7. Occupational Exposure to Bloodborne Pathogens; Final Rule. Federal Register. Part II; Department of Labor, Occupational Safety and Health Administration; 29 CFR Part 1910.1030; Friday, December 6, 1991.
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 9. Physician's Desk Reference, ed 49. Montvale, NJ, Medical Economics Data Production Company, 1995, pp 2184-2185.
 10. NCCLS Tentative Guideline EP5-T2. Precision performance of clinical chemistry devices - second edition. Villanova, PA: National Committee for Clinical Laboratory Standards, March 1992.
 11. NCCLS Proposed Guideline EP6-P. Evaluation of the linearity of quantitative analytical methods. Villanova, PA: National Committee for Clinical Laboratory Standards, August 1986.

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Appendix

COBAS MIRA® Instrument Settings**GENERAL**

MEASUREMENT MODE: ABSORB
 REACTION MODE : R-S-SR1
 CALIBRATION MODE: STD NONLIN
 REAGENT BLANK : NO BLANK
 CLEANER : NO
 WAVELENGTH : 340 nm
 DECIMAL POSITION: 0
 UNIT : ng/mL

ANALYSIS

SAMPLE DIL. NAME: H2O
 POST DIL. FACTOR: NO
 CONC. FACTOR: NO
 SAMPLE CYCLE: 1
 VOL: 36.0 µl DIL: 39.0 µL
 REAGENT CYCLE: 1
 VOL: 155 µL
 START REAGENT 1 CYCLE: 4
 VOL: 75.0 µL DIL: 20.0 µL

CALIBRATION

CALIB. INTERVAL: ON REQUEST
 STANDARD NONLINEAR CUP-POS: USER DEFINED
 1: 0 2: 50 ng/mL
 3: 100 4: 200 ng/mL
 5: 350 6: 500 ng/mL
 7: NO 8: NO
 REPLICATE : DUPL
 DEVIATION : NO
 CALC. MODEL : LOGIT/LOG4
 CORRECTION STD : NO

CONTROL

CS1 POS: NO
 CS2 POS: NO
 CS3 POS: NO

CALCULATION

SAMPLE LIMIT: NO
 REAC. DIRECTION: INCREASE
 CHECK: OFF
 ANTIGEN EXCESS: NO
 CONVERS. FACTOR: 1.00000
 OFFSET: 0.00000
 NORM. RANGE LOW: USER DEFINED*
 HIGH: USER DEFINED
 NUMBER OF STEPS : 1
 CALC. STEP A : KINETIC
 READINGS FIRST: 11 LAST: 22
 REACTION LIMIT: NO

*Note: the described sensitivity of the Emit® 2000 Cyclosporine Specific Assay is 40 ng/mL.

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COBAS MIRA S[®] and COBAS MIRA[®] Plus Instrument Settings

GENERAL

MEASUREMENT MODE: ABSORB
 REACTION MODE : R-S-SR1
 CALIBRATION MODE: LOGIT/LOG4
 REAGENT BLANK : NO BLANK
 CLEANER : NO
 WAVELENGTH : 340 nm
 DECIMAL POSITION: 0
 UNIT : ng/mL

CALIBRATION

CALIB. INTERVAL: ON REQUEST
 STANDARD POS: USER DEFINED
 1: 0 2: 50 ng/mL
 3: 100 4: 200 ng/mL
 5: 350 6: 500 ng/mL
 7: NO 8: NO
 REPLICATE : DUPL
 DEVIATION : NO
 CORRECTION STD : NO

ANALYSIS

POST DIL. FACTOR: NO
 CONC. FACTOR: NO
 SAMPLE CYCLE: 1
 VOLUME: 36.0 µL
 DILUTION NAME: H2O
 VOLUME: 39.0 µL
 REAGENT CYCLE: 1
 VOLUME: 155 µL
 START R1 CYCLE: 4
 VOLUME: 75.0 µL
 DILUTION NAME: H2O
 VOLUME: 20.0 µL

CONTROL

CS1 POS: NO
 CS2 POS: NO
 CS3 POS: NO

CALCULATION

SAMPLE LIMIT: NO
 REAC. DIRECTION: INCREASE
 CHECK: OFF
 ANTIGEN EXCESS: NO
 CONVERS. FACTOR: 1.00000
 OFFSET: 0.00000
 TEST RANGE LOW: ON
 HIGH: ON
 NORM. RANGE LOW: USER DEFINED*
 HIGH: USER DEFINED
 NUMBER OF STEPS : 1
 CALC. STEP A : KINETIC
 READINGS FIRST: 11 LAST: 22
 REACTION LIMIT: NO

**Note: the described sensitivity of the Emit[®] 2000 Cyclosporine Specific Assay is 40 ng/mL.*

[Pictogram
displayed below
in black & white]

Xn

Sodium azide [text in French, German, and Spanish]

R22 Harmful if swallowed. [Text in French, German, and Spanish]

R32 Contact with acids liberates very toxic gas.

S36 Wear suitable protective clothing. [Text in French, German, and Spanish]

Prepared in accordance with requirements for EEC label. [Text in French, German, and Spanish]

EINECS 247-852-1

European Distribution Center B.V.
Limpergstraat 4
Postbus 3272
2280 GG Rijswijk
Netherlands
Tel: 31 (0)70-395 09 14

Behring Diagnostics Inc.
Cupertino, CA 95014
1-800-227-8994 USA
1-800-267-6205 Canada

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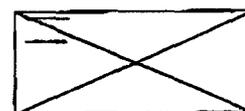
For additional assistance, call toll-free:

1-800-227-8994 USA

1-800-267-6205 Canada

***Notice: Adulteration of reagents,
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capabilities, or other failure to follow
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Cupertino, CA 95014**



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