Summary of Safety and Effectiveness

I.   General Information

Device Generic Name:	In Vitro reagent system for one the quantitative measurement of tacrolimus in human whole blood samples.
Device Trade Name:	PRO-Trac™ II Tacrolimus ELISA Kit
Applicant's name and address:	American Standard Companies DiaSorin, Inc. 9175 Guilford, Suite 100 Quarry Park Place Columbia, Maryland 21046
PMA number:	P970025
Date of panel recommendation:	none
Date of Good Manufacturing Practice Inspection:	November 13, 1998
Date of notice of approval to the applicant:	APR 2 7 1999

II.   Indications for Use

The PRO-Trac™ II Tacrolimus ELISA is an *in vitro* reagent system intended for the quantitative determination of tacrolimus (Prograf®, FK506) and some metabolites in EDTA or heparinized human whole blood as an aid in the management of liver transplant patients receiving tacrolimus therapy.

III.   Device Description

The DiaSorin PRO-Trac™ II Tacrolimus ELISA is a competitive enzyme immunoassay. Tacrolimus (FK506, Prograf®) is a macrolide lactone of fungal origin with strong immunosuppressive properties [1,2], and is used for the prevention of organ rejection. Tacrolimus has an in vitro potency 50-100 times greater than Cyclosporine A [2]. Despite its therapeutic properties, tacrolimus exhibits some toxicity. Its adverse effects resemble those of Cyclosporine A and include nephrotoxicity, gastrointestinal tract complaints, neurotoxicity, and glucose intolerance [3].

Tacrolimus is bound to the specific monoclonal antibody which is captured on the plate by the goat anti-mouse antibody. After a two-step incubation reaction, the chromogen reacts with the tacrolimus-horseradish peroxidase to produce a blue product. This reaction is stopped by addition of acid, which changes the color of the product to yellow, and the absorbance in each well is read at a dual wavelength of 450/630 nm. Color development is inversely proportional to the amount of tacrolimus present in the sample. Concentrations are interpolated from a standard curve.

Contraindications: There are no known contraindications for the PRO-Trac™ II Tacrolimus ELISA test.

Warnings: No firmly established therapeutic range exists for effective tacrolimus concentration in whole blood. Absorption and clearance of tacrolimus can vary greatly among patients. Clinical response to tacrolimus treatment does not correlate well with the administered dose, and whole blood concentrations of tacrolimus. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic and toxic effects of tacrolimus, coadministration of other immunosuppressants, time post transplant, and a number of other factors will result in different requirements for optimal blood levels of tacrolimus. Individual tacrolimus 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.

Precautions: Precautions for use of the device are found in the product labeling under "Warnings and Precautions" as well as " Limitations of the Procedure"

IV. Alternative Practices and Procedures

There are five general methods for the measurement of tacrolimus in whole blood. These methods include receptor binding, bioassay, High Pressure Liquid Chromatography (HPLC) with various detection methods, Microparticle Enzyme Immunoassay (MEIA), and enzyme-linked immunosorbent assay (ELISA) technologies.

V. Marketing History

The PRO-Trac™ II Tacrolimus ELISA is currently registered in France and Japan. Internationally, the kit has been sold in Canada, United Kingdom, Germany, Spain, Italy, Israel, and Japan. The PRO-Trac™ II ELISA has not been withdrawn from the market in any country.

VI. Adverse Effects of the Device on Health

A falsely elevated tacrolimus whole blood concentration could result in the physician lowering the tacrolimus dosage. As a result of the lowered dose of tacrolimus, the immunosuppressive ability of tacrolimus may not be effective enough to impede a cellular response, causing an adverse event of rejection. A falsely lowered tacrolimus whole blood concentration could result in the physician raising the tacrolimus dosage. As a result of the increased dose of tacrolimus, the patient may be subjected to an adverse event of toxicity.

VII. Summary of Studies

A. Non-Clinical Studies

Non-clinical laboratory studies for the evaluation of the PRO-Trac™ II Tacrolimus ELISA were conducted at DiaSorin Inc. facilities in Stillwater, Minnesota. HPLC/MS/MS analysis was conducted at Phoenix International, a private reference laboratory in Montreal, Canada. A summary of the technical performance claims shown in Table 1.

Table 1. Summary of Non-clinical Laboratory Studies

Parameter	Performance
Sensitivity [Minimum Detectable Limit]	0.27 ng/ml
Functional Sensitivity [CV% >20%]	1.0 ng/mL
Dilution Linearity Assay Range	1 - 30 ng/ml
Precision (internal study)	
Within Run [5 - 20 ng/ml]	< 10%CV
Total [5 - 20 ng/ml]	< 15%CV
Recovery	
By Standard Addition	81 ± 4% (Range 73 – 101%)
vs HPLC/MS/MS	114 ± 12% (Range 86 – 163%)
Cross-Reactivity (Metabolites)	
M I	0%
M II	85%
M III	38%
M IV	0%
M V	44%
M VI	0%
M VII	0%
Interfering Compounds	
Co-administered Drugs	*None Detected
Endogenous Compounds	
Triglycerides	*None
HAMA	*None
Uric Acid	* None
Bilirubin	Elevated levels may effect results
Protein	Elevated levels may effect results
Positional Drift	*None Detected
Reagent Stability	
Freeze/Thaw	4 Freeze/Thaw Cycles
Open Use	Stability to 180 Days
Sample Stability	
Freeze/Thaw	4 Freeze/Thaw Cycles
Room Temperature	Stability to 7 Days

* % error is within 15%.

Sensitivity

Sensitivity was determined for the PRO-Trac™ II ELISA both as an analytical sensitivity and as a functional sensitivity. Analytical sensitivity, 0.17 ± 0.06 ng/mL was extrapolated as the mean across three lots. This value is below concentrations encountered in clinical samples. Due to the curve fit employed for this assay, extrapolation to the analytical sensitivity is not valid [14]. Therefore, the functional sensitivity was determined as described below.

Functional Sensitivity [Minimum Detection Limit]

Functional sensitivity was defined as the concentration at which the analytical variation, expressed as %CV, exceeded 20%. Non-linear regression analysis of the precision of patient samples diluted into the range from 0.5 ng/mL to 2.0 ng/mL estimated 20% CV between 0.8 and 1.0 ng/mL. The functional sensitivity claim is 1.0 ng/mL.

Low concentration is also assessed by inter-assay precision by repeated assay of samples consisting of spiked whole blood samples of 1.0, 3.0, and 5.0 ng/mL. These samples were assayed as a single extraction per assay (see Table 3).

Table 3. Summary of Inter-Assay Precision

| Lot # | N | 1.0 ng/mL | | | 3.0 ng/mL | | | 5.0 ng/mL | | |
		Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
1	50	1.0	0.1	11.3	2.9	0.2	5.7	5.2	0.4	8.0
2	50	0.9	0.1	12.4	2.7	0.2	7.1	4.8	0.3	6.7
3	27	0.8	0.1	17.6	2.7	0.3	10.4	4.7	0.6	12.0
overall	127	0.9	0.1	15.2	2.8	0.2	8.6	4.9	0.5	9.3

Linearity of Dilution

Dilution linearity was assessed by diluting 3 high concentration spiked whole blood samples and 5 clinical samples with Zero Standard. The results were plotted as a linear regression of Expected versus Observed Values. The resulting linear regression was: $y = 0.90x + 0.21$ with $r = 0.990$. The resulting linear regression line for clinical samples was: $y = 0.99x + 0.27$ with $r = 0.996$. The regression line for all samples, spiked and clinical, assayed with all validation lots was $y = 0.95x + 0.3$ with $r = 0.991$. Based on this study the usable linear range for this assay is 1 to 30 ng/mL.

Precision

For precision determinations, spiked EDTA whole blood samples were prepared at nominal concentrations of 5.0, 10.0, and 20.0 ng/mL. These concentrations were chosen to reflect the recommended therapeutic range of 5 - 20 ng/mL as stated in the report from the Lake Louise Consensus Conference [5]. In addition, to examine the precision at very low concentrations inter-assay precision was determined using a range of controls from 1.0 to 5.0 ng/mL.

Within Run and Total Precision

Within-run and total precision of the PRO-Trac™ II ELISA was determined based on concepts from the NCCLS Precision Performance Guideline (EP5-T2). The precision study design incorporated multiple factors expected to influence precision. Duplicate precision samples at three concentration levels were tested in duplicate in 20 assays run over 20 operating days for each of three reagent lots. Within-run and total precision estimates were calculated for each lot and for all lots at the three concentration levels.

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An analysis of the components of variance was performed for the precision study using the SAS PROC VARCOMP (SAS 6.11).

A summary of the components of variance is listed in Table 4, and a summary of the precision performance evaluation is listed in Table 5. Taken together, the analytical precision data show a total imprecision of less than 10%.

Table 4. Components of Variance

Mean	3.9 ng/mL		8.2 ng/mL		16.5 ng/mL	
Component	SD	%CV	SD	%CV	SD	%CV
Extract	0.2	3.8	0.2	2.5	0.4	2.4
Day - Lot	0.2	5.2	0.5	5.6	1.1	6.8
Day	0.1	3.4	0.1	1.6	-	-
Lot	0.1	2.2	0.1	1.1	-	-
Total	0.3	**7.9%**	0.6	**6.8%**	1.3	**7.8%**

Table 5. Summary of the PRO-Trac™ II ELISA Precision (Internal Study)

5 ng/ml (N=80)

Lot	Mean	Within-Run	SD	%CV	Total	SD	%CV
1	3.9		0.3	6.3		0.3	6.9
2	3.8		0.3	7.5		0.3	8.8
3	4.0		0.2	4.7		0.3	7.0

10 ng/ml (N=80)

Lot	Mean	Within-Run	SD	%CV	Total	SD	%CV
1	8.3		0.4	5.2		0.5	5.8
2	8.0		0.5	6.5		0.6	7.2
3	8.2		0.5	5.7		0.6	7.1

20 ng/ml (N=80)

Lot	Mean	Within-Run	SD	%CV	Total	SD	%CV
1	16.5		1.0	5.8		1.1	6.5
2	16.3		1.4	8.7		1.6	9.8
3	16.8		1.0	5.6		1.2	6.8

Within Assay Precision

Within assay precision was evaluated using kit controls and EDTA whole blood samples spiked with tacrolimus at levels of 5.0, 10.0, and 20.0 ng/mL, respectively. Ten independent extractions were performed for each of the three concentration level samples or kit controls, and each extraction was run in duplicate in one assay.

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The within assay precision study resulted in %CVs for all samples and controls from three lots ranging from 1.9% to 6.2%. The within assay precision demonstrated %CVs less than 10%. The kit controls were also assayed at the clinical sites. The within assay precision demonstrated %CVs less than 15% at the clinical sites.

Recovery

Recovery was assessed using clinical samples which were diluted 1:2 with C, D, or E Standard and assayed in duplicate. Recovery for these samples was assessed against the HPLC/MS/MS values for the clinical samples and Kit Standards. The mean % recovery by this method was 114% ± 12% (range 86% - 163%).

In a second recovery design, known amounts of tacrolimus were added to an EDTA whole blood pool at a low concentration (5 ng/mL), an intermediate concentration (10 ng/mL) and a high concentration (20 ng/mL). The three tacrolimus concentration levels are within the suggested therapeutic range of tacrolimus as stated in the Lake Louise Consensus Conference Report [5]. Each of the three tacrolimus samples was run in ten replicate wells. The overall mean percent recovery by this method was 81% ± 4% (range 73% - 101%).

Tacrolimus Metabolite Cross-Reactivity

Cross reactivity was assessed by two methods. First, 5 ng/mL of each metabolite (MI-MVII) was added to the 5 ng/mL precision control and assayed. In a second assay, 5 ng/mL of metabolite was added to EDTA whole blood and assayed. The resulting cross-reactivities are consistent with published reports for this antibody [15] (see Table 6).

Table 6. Cross-Reactivity and Pharmacological Activity of Tacrolimus Metabolites

Metabolite	Modification	Cross-Reactivity (%)
M-I	(13-O-demethyl-)	0
M-II	(31-O-demethyl-)	84
M-III	(15-O-demethyl-)	36
M-IV	(12-O-hydroxyl-)	0
M-V	(15,31-O-didemethyl-)	42
M-VI	13,31-O-didemethyl-)	0
M-VII	(13,15-O-didemethyl-)	0
M-VIII	(31-O-demethyl, rearrangement)	ND

Interfering Compounds

Two general groups of compounds were examined for potential interference in the PRO-Trac™ II ELISA. The first group of compounds consisted of drugs commonly co-administered to transplant patients. The second group of compounds consisted of endogenous compounds which were elevated or depressed in clinical states which may be encountered in transplant patients.

Commonly Co-Administered Drugs

Interference by commonly co-administered drugs was examined by the addition of the test drug to the 5 ng/mL control material and assaying (N = 5) with an appropriate vehicle blank. Compounds were selected from previously published studies of interference with the original plasma-based ELISA developed by Fujisawa [20] and selected review of the transplant literature. Compounds were tested at levels approximately 2.5 times the expected therapeutic level, often 2 to 5 times the level tested in previous studies [20]. Compounds were considered to be interfering if the mean sample value was outside the expected range obtained with this precision control. The compounds and concentration tested are listed in Table 7.

Table 7. Assay Interference by Commonly Co-Administered Drugs

Drug	Concentration (µg/mL)	Mean Difference (5ng/mL control)
Acetaminophen	40	0.1
Acyclovir	5	0.1
Amikacin	60	-0.2
Amphotericin B	20	- 0.2
Azathioprine	1	0
Azithromycin	1	-0.3
Carbamazepine	20	0.2
Cefazolin	150	0.3
Ceftriaxone	500	0.2
Cimetidine	10	0.2
Clarithomycin	5	0
Cyclosporine	500	-0.1
Digoxin	5 ng/mL	0.1
Erythromycin	5	0.1
Famotidine	10	-0.1
Fluconazole	15	0.4
Furosemide	100	-0.3
Gentamicin	20	0.1
Ganciclovir	25	-0.3
Itraconazole	10	-0.1
Ketoconazole	10	-0.3
Lidocane	10	0
Mycophenolic Acid	600	-0.2
Methyl-Prednisolone	100	-0.1
Nifedipine	60	0.1
Penicillin	100	-0.3
Phenobarbital	80	0.3
Phenytoin	40	0.3
Prednisone	10	0.1
Rapamycin	5	-0.1
Ranitidine	1	-0.3
Tobramycin	20	0.2

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Trimethoprim	5	0.2
Sulfamethoxazole	150	-0.1
Valproic acid	200	0.1
Verapamil	1	-0.3

Four of these compounds were also examined at higher concentrations, spiked in patient samples. Acyclovir, (1000 μg/mL), Azithromycin, (5 μg/mL), Cyclosporine, (1000 μg/mL), and Gancilclovir(1000 μg/mL) were spiked into patient samples and analyzed as above. Using criteria that compounds are determined to be non-interfering if the mean test concentration was within 15% of the mean control concentration, none of the drugs listed exhibit interference in this assay at these higher concentrations.

Endogenous Interference

Interference by elevated triglycerides, bilirubin, protein (albumin), BUN, decreased hematocrit, and the presence of human anti-mouse antibody (HAMA) was examined. Only elevated protein and bilirubin showed an effect on assay results.

Analysis of Non-Drug Normals

Samples obtained from non-drug dosed normal individuals should read zero when assayed. Tacrolimus values were determined in non-dosed whole blood samples, and were found to be less than the A standard. These mean values were less than the calculated minimum detectable concentration [sensitivity] of the assay, and thus indistinguishable from zero.

Pharmacokinetic Studies

Pharmacokinetic studies were conducted on samples provided by Fujisawa USA (Deerfield, IL). Results are consistent with previously published studies [16].

Assay Drift Analysis

Drift was assessed due to positioning on the plate. For drift due to positioning, 5 sets of identical samples (In-house controls V-Z) were placed in multiple locations throughout the plate.

Lot-to-Lot Correlations

Clinical samples (n = 31) were assayed with each of three lots of kit components. The results were then compared between kit lots both by t test and by linear regression. When analyzed by t test the resulting p values no significant difference between the results obtained by the three different lots. When analyzed by linear regression, the correlation coefficients ranged from 0.984 to 0.991.

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Correlation to other methods: Clinical Samples

A subset of 150 clinical samples from 50 patients participating in the clinical study were analyzed by HPLC/MS/MS [10]. HPLC/MS/MS analysis was performed by Phoenix International (Montreal, Canada). Aliquots of these samples were also analyzed by PRO-Trac™ II ELISA at the clinical site. The resulting values were analyzed by linear regression. Linear regression analysis resulted in a line with the equation: ELISA = 1.1 (HPLC/MS/MS) + 0.5 with r = 0.905. Regression analysis of early post-transplant samples (within two weeks) gave a similar line [ELISA = 1.1 (HPLC/MS/MS) + 0.9; r = 0.94].

Verification of Standards/Controls/Spiked Samples

Standards, kit controls, and spiked in-house control samples were submitted to HPLC/MS/MS analysis as described above, with the results compared to the target values. Samples from all three validation lots were included in this analysis. Regression analysis of the Observed Value *vs* the Target Value resulted in a line with the equation Observed = 1.05 (Target) - 0.17, with r = 0.998.

Correlation to an Approved Device (IMx® Tacrolimus II MEIA)

A subset of 95 clinical samples (from 35 patients) were analyzed by Imx® tacrolimus II and Pro-Trac ™ II. These data were then compared by linear regression, resulting in a line equation: MEIA = 1.04 (ELISA) + 2.2; r = 0.89. Sample values which were not reported as a discreet value in either assay were not included in the regression analysis. The mean (± SD) difference between the values obtained by each method was –2.7 ± 2.3 ng/mL, with maximum difference to be –8.3 ng/mL or 6.1 ng/mL. On comparison of the same subset samples to HPLC/MS/MS, the mean difference were –0.8±2.3 ng/mL (m ± SD) with maximum differences -6.8 ng/mL and 4.2 ng/mL.

Reagent Stability

The stability of the reagents was examined in several studies. Real time stability was assessed as the ability of reagents in whole kit configurations to produce acceptable line parameters and acceptable control sample values. Open Use Stability was assessed on reagents in a whole kit configuration which had been opened, partially used, stored for defined intervals, and used for re-assay. All reagents were also subjected to multiple freeze/thaw cycles, and tested as a whole kit configuration after each freeze/thaw cycle.

Real Time Stability

Real time stability was tested on kit reagents stored at recommended temperatures. Kits were tested in whole system configurations only. Three validation lots were tested and initial shelf-life was established as 6 months.

Freeze/Thaw Stability

Reagents were subjected to 0 to 4 freeze/thaw cycles prior to testing. These reagents subsequently gave acceptable test results.

Open Use Stability

Open use stability was assessed on one lot. Kits were opened and assayed at Day Zero using approximately 1/3 of the device. The remaining reagents were stored consistent with the insert instructions and re-assayed at Day 30 and Day 180. Opened reagents stored for 0 to 180 days passed all quality control specifications.

Simulated Shipping Cycles

Kits from a validation lot were subjected to a simulated shipping cycle as described below. At the end of the cycle, these kits were tested according to the whole kit stability protocol. Reagents produced acceptable assay results, passing quality control specifications through Day 185.

Simulated Shipping Cycle Parameters:
        2-8°C for 20-24 hours
        43-47°C for 5 hours
        18-25°C for 20-24 hours
        35-39°C for 20-24 hours
        -15 to -25°C for 4-6 hours
        2-8°C for 20-24 hours
        Normal storage conditions

Sample Stability

Samples stability was demonstrated in several kinds of studies. Samples were assessed for the differences between fresh (within 72 hours) and frozen samples, the effects of repeated freeze/thaw cycles, and room temperature over a period of 7 days. Sample storage instructions in the package insert are consistent with these studies.

Heparin Anticoagulant Studies

To determine the acceptability of heparin as an anti-coagulant, split clinical samples were drawn in both EDTA and Heparin blood tubes, from 15 patients at the University of Pittsburgh clinical site. These samples were assayed at the clinical site and compared. When clinical specimens contain high tacrolimus levels (25-30 ng/mL), tacrolimus recovered from heparin tube can be 5.9 ng/mL higher than from an EDTA tube.

B. Clinical Studies

Summary of Clinical Study Design

A prospective, multicenter clinical study was conducted using the PRO-Trac™ II ELISA assay to measure the trough concentrations of tacrolimus in whole blood samples from

liver transplant patients receiving Prograf® as primary immunosuppressive therapy. With each subject, the three monitored primary clinical endpoints were:

- acute rejection, confirmed by histology
- nephrotoxicity
- any evidence of toxicity which required a reduction in Prograf® dosage

Two secondary clinical endpoints were also monitored; death and retransplantation due to graft failure. Additionally, mean changes from baseline were analyzed.

The objectives of this multicenter, open-label study were:

- To evaluate the PRO-Trac™ II ELISA system for measuring the level of tacrolimus in the whole blood of liver transplant subjects.

- To evaluate the relationship, if any, of measured tacrolimus blood levels to the subject's risk of rejection and toxicity.

- To validate the quality control procedures and assay precision of the PRO-Trac™ II ELISA system.

The study was conducted at six transplantation centers:

Emory University Hospital, Atlanta, GA	12 subjects
Mount Sinai Medical Center, NY, NY	18 subjects
University of Miami School of Medicine, Miami, FL	36 subjects
University of Pennsylvania Medical Center, Philadelphia, PA	15 subjects
University of Pittsburgh School of Medicine, Pittsburgh, PA	26 subjects
University of Wisconsin Hospital and Clinics, Madison, WI	4 subjects

Inclusion Criteria

Subjects were required to meet both criteria for inclusion in this study:
- Subject was eligible for allogenic liver transplant and would receive Prograf® (tacrolimus) as primary immunosuppressive therapy.
- Subject was able to fully execute the time and course of study participation.

Exclusion Criteria

Subjects meeting these criteria were excluded from the study:
- Subject was receiving a liver from an ABO incompatible donor.
- Subject had a prior organ transplant other than the liver, or subject was to undergo the transplantation of other organs at the time of liver transplantation.
- Subject was receiving an investigational immunosuppressant, with the exception of mycophenolate mofetil.

Study Population

One hundred and eleven (111) subjects were enrolled at 6 investigational sites. Thirty-nine (35.1%) subjects had histories of hepatitis C; 20 (18.0%) had histories of diabetes; 11 (9.9%) had histories of hepatitis B; and five (4.5%) were on dialysis. Reasons for transplantation were reviewed.

This study was conducted between August 1996 (date of first transplant) and April 1997 (three months follow-up after last subject transplant). Each subject underwent screening procedures prior to transplantation, and participated in the study for up to 12 weeks post-transplantation. Baseline demographic characteristics, age, gender, race, height, and weight were reviewed.

Ninety-one (82.0%) of the 111 enrolled subjects received Prograf® treatment for 12 weeks. Seventeen subjects (15.3%) received Prograf® treatment for less than 12 weeks because they reached clinical endpoints: Six (5.41%) were retransplanted due to graft failure and were not re-enrolled. Six (5.41%) experienced toxicity requiring reduction of Prograf® dosage. Three (2.7%) subjects died, and two (1.8%) subjects were re-transplanted due to graft failure and were re-enrolled in the study.

Three (2.7%) subjects were discontinued before receiving Prograf® treatment for 12 weeks, for reasons other than reaching clinical endpoints, (two (1.8%) due to changing the immunosuppressant to cyclosporine; and one (0.9%) due to lymphoma).

Clinical Performance Evaluation

Comparison of Tacrolimus Levels Using PRO-Trac™ II ELISA vs HPLC-MS/MS and Reference Device (Abbott IMx® MEIA)

The objective of this analysis was to correlate tacrolimus levels determined in whole blood with the PRO-Trac™ II ELISA, with values determined by HPLC/MS/MS and the reference device. A subset of 150 blood samples from 50 subjects was used to compare tacrolimus levels measured by the PRO-Trac™ II ELISA with HPLC/MS/MS. A subset of 95 of these samples also were analyzed by the reference device. To correlate results of the two methods to PRO-Trac™ II ELISA, samples were selected to represent time points across the 12 week monitoring window.

Clinical Sensitivity and Specificity

Since a therapeutic range could not be established in this study, calculations of clinical sensitivity and specificity do not apply. As a result, measured tacrolimus concentrations could not be utilized as the sole indicator for making changes in the treatment regimen.

Relationship of Prograf® Doses and Trough Tacrolimus Blood Concentrations

The relationship of Prograf® dose to tacrolimus trough concentrations in whole blood was analyzed. Although the analysis showed that the mean tacrolimus blood concentration was correlated to the mean Prograf® dose administered, the ability to predict through

concentrations based on dose is poor due to high inter- and intra-patient variability in absorption and bioavailability [2,5]. This indicated that direct measurement of tacrolimus concentrations in the patient's blood was required.

Quality Control

Quality control (QC) was performed in this study by specific assay and specimen criteria. Kit control samples with established acceptable ranges were used in every assay to ensure the validity of the obtained results. Assays or samples failing to meet the established criteria were repeated and replaced with valid results. QC acceptance criteria for the assay were:

1. Maximum absorbance of the zero standard was greater than 1.500.
2. Kit control values were within the ranges established by the manufacturer.
3. 4PL Parameter C was less than 5.0.

Sample results were considered acceptable if duplicate sample values varied no more than 20%.

QC kit control levels 1 and 2; maximum absorbance; and 4PL parameter C were consistently within the ranges established by the manufacturer, and maximum absorbance of the zero standard exceeded the criterion of >1.500, ranging from 2.000 to 2.700, with an across-site mean ($\pm$ SD) of 2.400 $\pm$ 0.370 and a coefficient of variation of 15.28. Across sites, the 4PL parameter C was < 5 for 94.4% of 195 samples.

Precision Analysis

The precision analysis data comprised 10 samples (PC1-PC10) analyzed at each of three sites on 20 randomly selected days. Two extracts were taken for each sample-site-day combination, with two duplicate wells for each extract. The data were unbalanced because only one extract was made at some site-day combinations. Within and total variability was assessed by the proficiency control samples and presented the N, mean, standard deviation, range, and CV%. Analyses were performed according to National Committee for Clinical Laboratory Standards, NCCLS document EP5-T2 (ISBN 1-56238-145-8).

Within site variability across all levels (encompassing day to day and lot to lot variability) averaged 12%; total reproducibility (all sites) averaged 14%. The greatest components of variation from the multi-way ANOVA appear to be day to day, followed by site to site variation. Within site and total reproducibility are summarized in Table 8; estimates of the components of variation from multi-way ANOVA are summarized in Tables 9.

Table 8. Within and Between Site Reproducibility

Sample #/ Parameter	Mount Sinai	University of Pittsburgh	University of Pennsylvania	Total
N (PC 1)	34	50	40	124
Mean ± SD	13.4 ± 1.4	12.4 ± 1.3	11.0 ± 1.4	12.2 ± 1.6
%CV	10.3%	10.5%	12.4%	13.4%
N (PC 2)	33	54	39	126
Mean ± SD	17.3 ± 1.8	15.6 ± 1.6	14.9 ± 1.5	15.8 ± 1.9
%CV	10.6%	9.9%	10.3%	11.8%
N (PC 3)	33	52	40	125
Mean ± SD	9.7 ± 1.0	8.6 ± 1.0	7.5 ± 1.0	8.5 ± 1.3
%CV	10.3%	11.8%	13.3%	15.3%
N (PC 4)	36	55	38	129
Mean ± SD	4.0 ± 0.6	3.8 ± 0.5	2.9 ± 0.5	3.6 ± 0.7
%CV	14.7%	14.2%	16.6%	19.4%
N (PC 5)	36	56	34	126
Mean ± SD	5.2 ± 0.9	5.2 ± 0.6	4.2 ± 0.5	4.9 ± 0.8
%CV	17.1%	12.5%	11.1%	16.6%
N (PC 6)	36	54	38	128
Mean ± SD	8.3 ± 1.1	7.9 ± 0.9	6.5 ± 0.9	7.6 ± 1.2
%CV	14.0%	11.1%	13.6%	15.9%
N (PC 7)	34	55	39	128
Mean ± SD	12.9 ± 1.6	12.6 ± 1.4	11.5 ± 1.5	12.3 ± 1.6
%CV	12.2%	10.7%	13.1%	12.7%
N (PC 8)	36	52	40	128
Mean ± SD	10.4 ± 1.2	9.9 ± 1.0	8.4 ± 0.9	9.6 ± 1.3
%CV	11.1%	9.6%	10.2%	13.3%
N (PC 9)	35	52	40	127
Mean ± SD	10.8 ± 1.4	10.5 ± 1.1	9.3 ± 1.0	10.2 ± 1.3
%CV	13.0%	10.1%	10.7%	12.6%
N (PC 10)	34	52	40	126
Mean ± SD	17.7 ± 1.9	17.2 ± 1.8	15.4 ± 1.6	16.8 ± 2.0
%CV	10.8%	10.4%	10.1%	11.9%

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Table 9. Estimates of Components of Variation from Multi-way ANOVA

Sample	Site to Site	Day to Day Within Site	Extract to Extract Within Day	Measurement Error
PC 1	1.109	1.682	0.000	0.444
PC 2	0.966	2.065	0.000	1.171
PC 3	0.891	0.711	0.000	0.568
PC 4	0.268	0.221	0.023	0.079
PC 5	0.247	0.333	0.000	0.213
PC 6	0.675	0.801	0.000	0.347
PC 7	0.335	1.943	0.235	0.085
PC 8	0.868	1.000	0.184	0.000
PC 9	0.419	1.133	0.000	0.367
PC 10	1.105	2.888	0.567	0.000

Clinical Summary

In this study, whole blood tacrolimus levels were evaluated with the PRO-Trac™ II ELISA system in 111 liver transplant patients receiving Prograf® as primary immunosuppressive therapy. Over the 12 week post-transplant evaluation period, incidence rates for monitored primary clinical endpoints were: acute transplant rejection (confirmed by histology), 36 subjects (32.4%); nephrotoxicity, defined as increased serum creatinine levels at least twofold greater than baseline values, 38 subjects (34.2%); and any toxicity requiring a reduction in Prograf® dosage, 10 subjects (9.0%). Incidence rates over the 12 week period for monitored secondary clinical endpoints were: death, 3 subjects (2.7%); and re-transplantation due to graft failure, 8 subjects (7.2%).

Although the analysis showed that the mean tacrolimus blood concentration was correlated to the mean Prograf® dose administered, the ability to predict trough concentrations based on dose is poor due to high inter- and intra-patient variability in absorption.

The relationship between the whole blood tacrolimus levels and adverse events could not be established by this study.

The monitoring of whole blood through concentrations of tacrolimus with the PRO-Trac™ II ELISA system provided information of value as an aid in managing liver transplant patients receiving tacrolimus therapy.

The quality control procedures and assay precision of the system performed within protocol-defined parameters for within-site and total reproducibility.

VIII. Conclusions Drawn from the Studies

The analytical studies demonstrated that the PRO-Trac™ II ELISA provided acceptable performance in the areas of precision (<20%), analytical and functional sensitivity

(1 ng/mL), dilution linearity, tacrolimus recovery (20% bias), cross-reactivity, susceptibility to interfering substances, and drift. These studies also indicated that tacrolimus levels above the standard curve may be acceptably diluted into the standard range to produce reportable results. Total and within-run precision values were within clinically acceptable ranges (< 20%). These studies also demonstrated acceptable correlation (r = 0.9) to an alternative tacrolimus measurement method (HPLC/MS/MS).

The clinical studies demonstrated that the PRO-Trac™ II ELISA provides tacrolimus values in liver transplant patients receiving Prograf®. The data presented from the analytical and clinical studies demonstrate that the PRO-Trac™ II ELISA is safe and effective for its intended use when used in accordance with the instructions provided in the product insert. The measured level by this method cannot be used as the sole indicator for making changes in the treatment regimen.

IX. Panel Recommendation

Pursuant to section 515 (c) (2) of the act as amended by the safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Clinical Chemistry and Clinical Toxicology Devices Advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel.

X. CDRH Action

CDRH issued an approval order for the applicant's PMA for the PRO-Trac™ II Tacrolimus ELISA on April 27, 1999.

The applicant's manufacturing facilities were inspected on November 13, 1998, and were found to be in compliance with the Good Manufacturing Practice Regulations (GMPs). The shelf-life of the PRO-Trac™ II Tacrolimus ELISA has been established at 6 months.

XI. Approval Specification

Directions for use: See 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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