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Emit® 2000 Tacrolimus Assay

See shaded sections:
Updated information from 2018-11 edition.

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Tacrolimus Assay

1 Intended Use

The Emit® 2000 Tacrolimus Assay is for *in vitro* quantitative analysis of tacrolimus and metabolite in human whole blood as an aid in the management of tacrolimus therapy in liver and kidney transplant patients.

2 Summary and Explanation of the Test

Tacrolimus, discovered by Fujisawa Pharmaceutical Co, Ltd in 1984, is a macrolide immunosuppressant of fungal origin.[1,2] Tacrolimus is a calcineurin inhibitor with selective action on T lymphocytes.[3–5]

Extensive data have confirmed the efficacy of tacrolimus as a primary or rescue immunosuppressant.[2] Tacrolimus has a narrow range of safe and effective therapy.[3,6] Inadequate tacrolimus dosages or levels may result in transplant rejections.[3] Toxic levels of tacrolimus are associated with many side effects including nephrotoxicity, neurotoxicity, post transplant diabetes, susceptibility to infections, malignancies, hypertension, and gastrointestinal disturbances.[2,4,7]

The most effective means of ensuring adequate immunosuppression therapy for solid organ transplant is monitoring tacrolimus concentrations in whole blood and interpreting the concentrations in conjunction with other laboratory data and clinical considerations.[4,5] The clinical response to tacrolimus therapy may not correlate well with administered dose.[1,5,7] Both absorption and clearance of tacrolimus vary greatly among patients.[4] Factors affecting tacrolimus concentrations include the age of the patient, nature of the transplant, preexisting health conditions, and the coadministration of tacrolimus with other drugs.[4–7]

Tacrolimus is metabolized by way of the cytochrome P450 pathway. Several drugs that induce the cytochrome P450 enzymes are known to lower tacrolimus concentration. Many drugs are known to increase tacrolimus levels in the blood.[6,7] Therefore, variations in tacrolimus blood levels may be observed if the drug regimen of the patient is changed. See Section 9, Expected Values.

The Consensus Document for tacrolimus recommends whole blood collected with EDTA as the preferred specimen matrix.[4] The contribution of tacrolimus metabolites to immunosuppression or toxicity remains uncertain.[3,4,6] Currently available immunoassays are nonspecific due to cross-reactivity with metabolites and may overestimate the concentration of tacrolimus. In such cases, the use of a specific assay (e.g. HPLC/MS) to measure tacrolimus could be considered.[3,5]

Other methods used to monitor tacrolimus concentrations include an ELISA, microparticle enzyme immunoassay (MEIA), high-performance liquid chromatography (HPLC), and high-performance liquid chromatography/tandem mass spectroscopy (HPLC/MS/MS).[3]

3 Principle

The Emit® 2000 Tacrolimus Assay employs a homogeneous enzyme immunoassay technique used for analysis of tacrolimus in whole blood. This assay contains mouse monoclonal antibodies with a high specificity for tacrolimus.

The Emit® 2000 Tacrolimus Assay is based on competition for tacrolimus antibody binding sites. Tacrolimus in the sample competes with tacrolimus in the Enzyme Reagent that is labeled with recombinant enzyme glucose-6-phosphate dehydrogenase (rG6PDH). Active (unbound) rG6PDH enzyme converts the oxidized nicotinamide adenine dinucleotide (NAD) in the Antibody Reagent to NADH, resulting in a kinetic absorbance change that can be measured spectrophotometrically. Enzyme activity decreases upon binding to the antibody, allowing tacrolimus concentrations to be measured in terms of enzyme activity. Endogenous serum G6PDH does not interfere with the assay because coenzyme NAD functions only with bacterial (*Leuconostoc mesenteroides*) enzyme employed in this assay.

Pretreatment Step

Before testing with the Emit® 2000 Tacrolimus Assay, whole blood samples, calibrators, and controls are pretreated with methanol and the Emit® 2000 Sirolimus/Tacrolimus Sample Pretreatment Reagent. The pretreatment process lyses the cells, extracts the tacrolimus, and precipitates most of the blood proteins. The pretreated samples are centrifuged, and an aliquot of the resulting supernatant containing tacrolimus is then assayed using the Emit® 2000 Tacrolimus Assay.

4 Reagents

REF	Product Description	Quantity/Volume
8R019UL	**Emit® 2000 Tacrolimus Assay**	
	Antibody Reagent 1 Mouse monoclonal antibody reactive to tacrolimus (4.4 µg/mL),* NAD (18 mM), G6P (22 mM), sodium chloride, bovine serum albumin, surfactant, and preservatives	22 mL
	Buffer Reagent 2 Tris buffer, bovine serum albumin, surfactant, and preservatives	10 mL
	Enzyme Reagent 3 Tacrolimus labeled with bacterial rG6PDH (0.31 U/mL),* phosphate buffer, bovine serum albumin, and preservatives	10 mL

**** The antibody titer and enzyme conjugate activity may vary from lot to lot.***

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

Warning

- Handle all specimens as if capable of transmitting infection.
- Do not eat, drink, smoke, or apply cosmetics where reagents, calibrators, and controls are being handled.
- Do not pipette by mouth.
- Wear disposable gloves and appropriate laboratory protective apparel while handling samples and calibrators. Thoroughly wash hands afterwards.
- Always handle human derived materials as if they were potentially infectious. The calibrators contain human blood components. Each lot is tested and found to be nonreactive for Hepatitis B Surface Antigen (HBsAg), antibody to Human Immunodeficiency Virus (HIV 1/2), antibody to Hepatitis C Virus (HCV), and antibody to syphilis.
- Methanol, which is a material required but not provided for the Emit® 2000 Tacrolimus Assay and is used to extract tacrolimus from samples, is flammable. Do not ingest. Avoid skin contact.

For *in vitro* diagnostic use.

Preparation, Storage, and Stability of Assay Components

Reagents

The Emit® 2000 Tacrolimus Assay reagents are provided ready for use.* Close the reagent bottles when not in use. Always return the reagent screw caps to their original containers.

**** If the antibody reagent turns yellow, it has deteriorated and, therefore, must be discarded along with its accompanying buffer and enzyme reagents.***

Do not freeze reagents or expose them to temperatures above 32°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.

5 Specimen Collection and Handling

- The required sample volume is 200 µL of whole blood.
- Blood should be drawn using tubes containing the anticoagulant EDTA. EDTA is recommended as the anticoagulant of choice for assaying tacrolimus in whole-blood samples. Heparinized samples are not recommended because they may form clots during storage.[4]
- 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 1 week. If longer storage is necessary samples should be frozen at -20°C. Tacrolimus has been shown to be stable in whole-blood samples for at least 6 months when stored at -20°C.[4] 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.
- 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 tacrolimus.[6]
- All specimens should be handled as if capable of transmitting disease. Follow standard precautions for handling infectious agents during all procedures.[8]

6 Procedure

Materials Provided

Emit® 2000 Tacrolimus Assay

Antibody Reagent 1
Buffer Reagent 2
Enzyme Reagent 3

Materials Required But Not Provided

8R109UL Emit® 2000 Tacrolimus Calibrators
8S079UL Emit® 2000 Sirolimus/Tacrolimus Sample Pretreatment Reagent
Methanol (ACS Reagent/HPLC grade)
Multilevel whole-blood controls
Positive displacement pipettes:

- 200 µL pipette or Eppendorf Repeater (2.5 mL or 5 mL Combitip or equivalent for delivery of methanol)
- 50 µL pipette or Eppendorf Repeater (1.25 mL Combitip or equivalent for delivery of Pretreatment Reagent)
- 100 µL or 200 µL pipette (Rainin Microman M250 or equivalent for dispensing samples)

Eppendorf Combitips (1.25, 2.5, or 5 mL) if Eppendorf Repeater is used
Microcentrifuge tubes (1.5 to 2 mL capacity)
Microcentrifuge
Microcentrifuge tube rack
Vortex mixer
Laboratory tissues
Sample inverter or rocker (optional)

Setup Procedure

1. Allow calibrators, controls, and samples to stand at ambient or room temperature (18–25°C) before use.
2. Use the Emit® 2000 Sirolimus/Tacrolimus Sample Pretreatment Reagent and methanol at room temperature (18–25°C). Store at 2–30°C.
3. Prepare, store, and use controls according to manufacturer's directions.
4. Set up and label 1 microcentrifuge tube for each calibrator, control, and sample to be pretreated.

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: 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 7, 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.

Do not pretreat more than 30 samples at one time.

STEPS	TECHNICAL NOTES
1. Mix all calibrators, controls, and samples gently but thoroughly just before use.	• **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.
2. Transfer 200 µL of each calibrator, control, and/or sample to the appropriately labeled microcentrifuge tube using a positive displacement pipette.* (See Note at Step 5)	• A single capillary tube (Rainin Microman M250 or equivalent) 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.
3. Add 200 µL of methanol to each microcentrifuge tube with a positive displacement pipette.* (See Note at Step 5)	• A positive displacement pipette or Eppendorf Repeater (Combitip 2.5 mL or 5 mL) must be used for dispensing methanol because the viscosity of methanol is low relative to that of water.
4. Add 50 µL of Sample Pretreatment (cupric sulfate) to each microcentrifuge tube with a positive displacement pipette. **Immediately** cap each tube.* (See Note at Step 5)	• A positive displacement pipette or Eppendorf Repeater (Combitip 1.25 mL) is recommended. Some control matrices may gel quickly if cupric sulfate is added prior to methanol.
5. Vortex each microcentrifuge tube for at least 10 seconds. **** Steps 2 to 4 may be reversed for narrow conical tubes in order to aid vortex mixing of sample.***	• Vortexing soon after adding the methanol and cupric sulfate will minimize the time needed to break up any pellets that may form. Sample mixture should be completely homogeneous immediately after vortexing.
6. Incubate the contents of the microcentrifuge tubes at room temperature (18–25°C) for at least 1 minute after vortexing of the last sample is completed.	• Microcentrifuge tubes may incubate up to 1 hour after vortexing and before centrifuging.
7. Spin the microcentrifuge tubes in a microcentrifuge for at least 2 minutes.	• If the supernatant is cloudy or becomes cloudy on standing, it should be recentrifuged (g force x minutes ≥ 25,000 g-min).
8. Decant the supernatant into the appropriate sample cup/tube. Samples are ready to be assayed.	• To guard against obtaining an assay value when no sample is present, ensure that all sample cups contain sample.

Calibration

1. Calibrate whenever a new lot of reagents is used. Recalibration may be necessary when significant changes to the instrument (e.g. 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 operators' manual.
3. Pretreat a set of Emit® 2000 Tacrolimus Calibrators according to the sample pretreatment protocol. Calibrators may be pretreated along with samples and controls.
4. Place the pretreated calibrators in the appropriate sample position/rack and calibrate according to instructions in the 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 bi-level or tri-level controls in a single run. 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 (9) results at each level must be recorded from the 3 runs.
3. Calculate a mean control concentration for each control level based on the 9 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 1.

Table 1 — Control Limits

Control	Mean Control Concentration (ng/mL)	Limit
Low	3–7	mean ± 30%
Medium	10–14	mean ± 20%
High	20–25	mean ± 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 the Technical Assistance Center.

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. **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 9 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 ±12% of the mean and not greater than ±25% of the mean. If ±2.25 SD of the mean is less than ±12% of the mean, set the *permanent* control limits at ±12% of the mean. If ±2.25 SD of the mean is greater than ±25% of the mean, set the permanent control limits at ±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

Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of a Quality Control (QC) material with known tacrolimus concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.

Diluting High-Concentration Samples

If a pretreated patient sample assays higher than 30 ng/mL tacrolimus use the following directions to manually dilute the original sample.

High-concentration, whole-blood samples may be diluted with either the negative whole-blood calibrator or negative EDTA whole blood using the following directions:

1. Mix **negative whole-blood** and **high-concentration whole-blood** sample gently but thoroughly just before use.
2. Combine 1 part **high-concentration whole-blood** sample with 2 parts **negative whole-blood sample.**
3. Mix the diluted whole-blood sample thoroughly by repeated inversion.
4. Pretreat and assay the diluted whole-blood sample using steps 1 through 8 of the Pretreatment and Assay Procedure.
5. Multiply the assay result by the dilution factor to obtain an estimate of the tacrolimus concentration.

7 Results

Results are calculated automatically by the analyzers. No additional manipulation of the data is required unless samples have been manually diluted.

This assay uses Math Model No. 1.

Consult the appropriate instrument operating manual and analyzer-specific application sheet for complete instructions.

8 Limitations

The Emit® 2000 Tacrolimus Assay is for *in vitro* diagnostic use in the measurement of tacrolimus in whole blood. This assay is not intended to be used for measuring tacrolimus in serum or plasma.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Siemens Healthcare Diagnostics has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these instructions for use.

Microbial contamination of the analyzer may cause tacrolimus enzyme carryover. This may elevate the sample results obtained for tacrolimus and other Emit® assays run immediately after a tacrolimus run. The observed impact varies and is assay-dependent. For more information, please contact your Siemens representative.

Venipuncture should occur prior to sulfasalazine and/or sulfapyridine administration due to the potential for falsely elevated results.

Warning: Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits that utilize mouse monoclonal antibodies. These specimens should not be assayed with the Emit® 2000 Tacrolimus Assay.

9 Expected Values

No firm therapeutic range exists for tacrolimus in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of tacrolimus, time of sample collection, 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 tacrolimus. Individual tacrolimus values cannot be used as the sole indicator for making changes in treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made and each user must establish their own ranges based on clinical experience. Therapeutic ranges vary according to the commercial test used, and therefore should be established for each commercial test. Values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and cross-reactivity with metabolites, nor should correction factors be applied. Therefore, consistent use of one assay for individual patients is recommended.

The Consensus Document has described targets for 12-hour trough whole-blood concentrations.[4] They range from 5 to 20 ng/mL, depending on the transplant type, stage after transplantation, and the medical practice.[3] Higher or lower concentrations may be associated with an increase in the incidence of adverse effects.

Blood levels can be affected by co-medications. Patients treated with viral protease inhibitors for HIV infection may have dramatically altered metabolism of tacrolimus, which may cause elevation of tacrolimus to at least 100 ng/mL, and may require novel dosing.[9]

The following information, reported by Fujisawa Pharmaceutical Co, Ltd is taken from the Physicians' Desk Reference.[10] It includes the co-medications that may increase or decrease blood levels of tacrolimus, but the advent of new co-therapies may add to this list.

Tacrolimus is extensively metabolized by the liver. Therefore, circulating tacrolimus levels may be influenced by drugs that affect hepatic microsomal enzymes, particularly the cytochrome P450 system. Substances known to inhibit these enzymes will decrease hepatic metabolism and increase tacrolimus levels.

Substances that are inducers of cytochrome P450 activity will increase hepatic metabolism and decrease tacrolimus levels. Monitoring of circulating tacrolimus levels and appropriate Prograf® (tacrolimus) dosage adjustment is essential when these drugs are used concomitantly.

Drugs That May *Increase* Tacrolimus Levels

Bromocriptine	Diltiazem	Nicardipine
Cimetidine	Erythromycin	Nifedipine
Cisapride	Fluconazole	Protease Inhibitors
Clarithromycin	Itraconazole	Troleandomycin
Clotrimazole	Ketoconazole	Verapamil
Cyclosporine	Methylprednisolone	
Danazol	Metoclopramide	

Drugs That May *Decrease* Tacrolimus Levels

Carbamazepine	Phenytoin	Rifampin
Phenobarbital	Rifabutin	

10 Specific Performance Characteristics

Performance characteristics of the Emit® 2000 Tacrolimus 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 Tacrolimus Assay quantitates tacrolimus concentrations in human whole blood containing 2.0 to 30 ng/mL tacrolimus. Quantitative results above 30 ng/mL and up to approximately 80 ng/mL or greater can be estimated by diluting and reassaying high-concentration samples and multiplying the results by the dilution factor (see Section 6, Procedure, Diluting High-Concentration Samples). Dilutional recovery was studied up to 80 ng/mL, and the diluted tacrolimus value was within 10% of the expected concentration. No carryover from High (80 ng/mL) to Low (5 ng/mL) samples or from Low to 24 ng/mL samples occurred.

Sensitivity

The sensitivity of the Emit® 2000 Tacrolimus Assay is 2.0 ng/mL. This is the lowest concentration of tacrolimus that can be distinguished from 0 ng/mL with a confidence level of 95%.

Functional Sensitivity

The functional sensitivity of the Emit® 2000 Tacrolimus Assay was determined by assaying specimens and by assessing the interassay precision for these specimens. Four (4) patient sample pools (values from 0.0–3.0 ng/mL) were measured once a week for six weeks with two different lots of reagents and calibrators. The limit of quantitation at 20% CV was 2.8 ng/mL.

Specificity

The cross-reactivity of 8 available major tacrolimus metabolites was evaluated in the presence of 0 ng/mL tacrolimus. Cross-reactivity was determined by dividing the apparent tacrolimus concentration by the concentration of added metabolite and expressing the result as a percentage. The apparent tacrolimus concentration was determined by subtracting the actual tacrolimus concentration (0 ng/mL) from the measurement for tacrolimus. Results are shown in Table 2.

Table 2 — Metabolite Cross-Reactivity in the Presence of Tacrolimus

Tacrolimus Metabolite	Level Tested (ng/mL)	Cross-reactivity (%)
M-1 (13-O-demethyl tacrolimus)	100	10.4
M-2 (31-O-demethyl tacrolimus)	100	1.6
M-3 (15-O-demethyl tacrolimus)	100	2.2
M-4 (12-hydroxy tacrolimus)	100	21.1
M-5 (15, 31-O-didemethyl tacrolimus)	100	2.5
M-6 (13, 31-O-didemethyl tacrolimus)	100	0.6
M-7 (13, 15-O-didemethyl tacrolimus)	100	0.9
M-8	100	2.2

Compounds whose chemical structure or concurrent therapeutic use would suggest possible interference have been tested. The compounds listed in Table 3 did not interfere with the Emit® 2000 Tacrolimus Assay when tested in the presence of 15 ng/mL tacrolimus. Levels tested were determined according to the Clinical and Laboratory Standards Institute (CLSI) [formerly named National Committee for Clinical Laboratory Standards (NCCLS)] EP7-P.[11] Systemic inaccuracies (bias) due to these substances are less than 10% at tacrolimus concentration of 15 ng/mL.

Table 3 — Co-Administered Drugs and Drug Metabolites That Do Not Interfere

Drug	Test Level (µg/mL)	Drug	Test Level (µg/mL)
Acetaminophen	200	Isoproterenol Hydrochloride	0.06
N-Acetylprocainamide	120	Kanamycin	100
Acyclovir	1000	Ketoconazole	70
Albuterol	0.18	Labetalol	200
Allopurinol	60	Lidocaine	100
Alprazolam	0.37	Lovastatin	4
Amikacin	150	Metformin HCl	5100
Amitryptyline	20	Methylprednisolone	12
Amphotericin B	100	Metoclopramide	4
Ascorbic Acid	30	Misoprostol	0.015
Atenolol	40	Morphine Sulfate	6
Azathioprine	10	Muromonab-CD3	1
Caffeine	100	Mycophenolic Acid	400
Captopril	50	Mycophenolic Acid Glucuronide	1000
Carbamazepine	120	Nadolol	480
Cefaclor	230	Naproxen	1000
Chloramphenicol	250	Niacin	12000
Cimetidine	100	Nifedipine	120
Ciprofloxacin	43	Nitroglycerin	5
Cyclophosphamide	250	Omeprazole	14
Cyclosporin A (CsA)	1	Penicillin G	100
CsA AM1 Metabolite	0.5	Phenobarbital	150
CsA AM19 Metabolite	0.5	Phenytoin	100
CsA AM4N Metabolite	0.5	Piperacillin	8
CsA AM9 Metabolite	0.5	Prazosin	25
Digoxin	10	Prednisolone	12
Dipyridamole	25	Prednisone	12
Disopyramide	30	Primidone	100
Doxazosin	32	Procainamide HCl	25
Erythromycin	200	Promethazine	10
Ethanol	3500	Propranolol	0.5
Fluconazole	81	Quinidine Sulfate	100
Furosemide	100	Ranitidine	200
Ganciclovir	400	Rapamycin	0.375
Gentamicin	120	Rifampin	50
Glipizide	60	Salicylic Acid	500
Glyburide	40	Spectinomycin	100
Heparin	8000 U/L	Sulfamethoxazole	400
Hydralazine	32	Theophylline	250
Hydrochlorothiazide	40	Triamterene	600
Ibuprofen	400	Trimethoprim	20
Indomethacin	10	Valproic Acid	1000
Insulin	400 µU/mL	Vancomycin	630
Isoniazid	70	Verapamil	10

Endogenous Substances

Albumin, bilirubin, uric acid, triglyceride (Intralipid®) or cholesterol was added to tacrolimus EDTA whole blood patient pool. Systemic inaccuracies (bias) due to these substances are less than 10% at tacrolimus concentration of 15 ng/mL.

Compound	Concentration Tested
Albumin	12 g/dL
Bilirubin	60 mg/dL
Cholesterol	500 mg/dL
Triglyceride (Intralipid®)	1500 mg/dL
Uric Acid	26 mg/dL
IgG Gamma Globulin	12 g/dL
Rheumatoid Factor	500 IU/mL

Other potential interferences

No method-to-method interference occurred among the Emit® assays for cyclosporine, mycophenolic acid, or tacrolimus when run on the V-Twin® analyzer.

The presence of heparin does not interfere with the measurement of tacrolimus, although the use of heparin as an anticoagulant is discouraged due to the potential for the formation of microclots.

Precision

Total precision and its within-run precision component were determined according to the CLSI/NCCLS guideline for evaluation of precision.[12] Spiked human blood hemolysate pools were tested in duplicate, 2 runs per day for 20 days (N = 80). Total precision was determined on the V-Twin® analyzer. Table 4 shows the mean value for each control level, the standard deviation (SD), and the coefficient of variation (%CV) obtained.

Table 4 — Precision

Control Level (ng/mL)	Within-run Precision SD	Within-run Precision %CV	Total Precision SD	Total Precision %CV
5.10	0.40	7.8	0.83	16.4
9.98	0.50	5.0	1.03	10.3
15.11	0.59	3.9	1.27	8.4
23.87	0.81	3.4	1.62	6.8

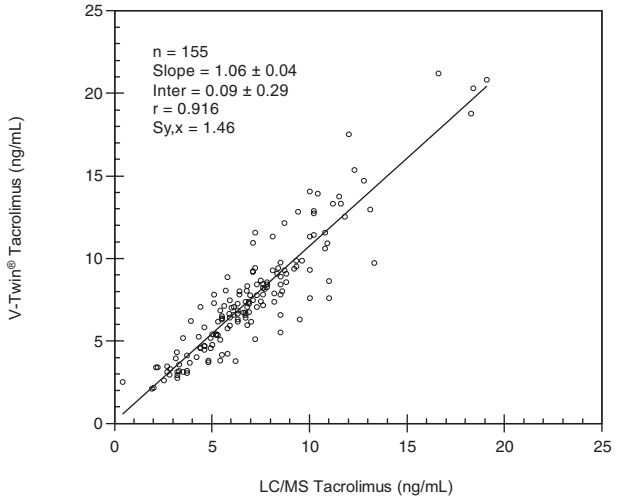
Method Comparison

Evaluations were conducted at two external sites, with samples collected from two transplant patient groups (renal and liver.) The patient samples were analyzed on the V-Twin® and by a Liquid Chromatography/ tandem Mass Spectrophotometer (LC/MS/MS) method and the Abbott IMx® (employing a specific monoclonal antibody). A comparative analysis of the results is shown in Table 5.

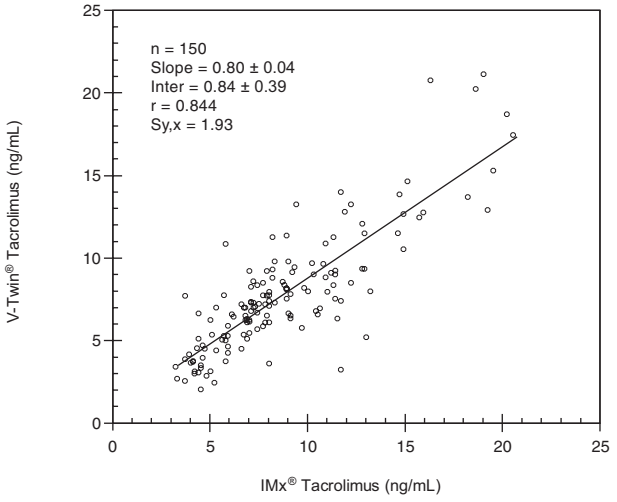
Table 5 — Method Comparison

Comparative Method	Slope	Intercept	R	n
LC/MS/MS				
All	1.06	0.09	0.916	155
Liver	1.01	0.35	0.946	65
Kidney	1.10	-0.12	0.899	90
Abbott IMx®				
All	0.80	0.84	0.844	150
Liver	0.83	0.69	0.812	62
Kidney	0.79	0.87	0.863	88

Tacrolimus Method Comparison Combined Site Liver & Kidney



Tacrolimus Method Comparison Combined Site Liver & Kidney



Recovery

Tacrolimus recovery was assessed using tacrolimus-free EDTA whole blood to which known amounts of tacrolimus were added. Whole blood was spiked at 5 ng/mL, 15 ng/mL, and 25 ng/mL. Four separate runs of each spike were pretreated and assayed five times. Recovery study results are shown in table 6.

Table 6 — Recovery Study

Nominal Value (ng/mL)	5.0	15	25
Mean Measured Values (ng/mL)	5.3	16.1	27.6
Percent Recovery	106%	107%	110%

A separate study was conducted to determine the effect of hematocrit on the recovery of tacrolimus from a sample. Red blood cells were added to samples to create five whole-blood samples with hematocrits ranging from 15% to 60%. Nominal concentration of 15 ng/mL tacrolimus was added to separate aliquots of each of the samples. These aliquots were then assayed. The effect of hematocrit on the recovery of tacrolimus was within 10% of the control sample for samples ranging from 15% to 60% hematocrit.

Linearity

The linearity of the Emit® 2000 Tacrolimus Assay was assessed according to the CLSI/NCCLS guideline for evaluation of linearity.[13] The assay was found to be linear within the range 2 to 30 ng/mL. The information in Table 7 was determined by plotting target value (x) versus analytical result (y).

Table 7 — Linearity Study

Slope	1.024
Intercept	0.244
r	0.9992

11 Risk and Safety

H317
P280, P272, P302 + P352, P333 + P313, P501
Warning!
May cause an allergic skin reaction.

Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone

Safety data sheets (MSDS/SDS) available on siemens.com/healthcare

12 Bibliography

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13 Symbols Key

Notes

For technical assistance, call Siemens Healthcare Diagnostics:
1-800-227-8994 in the USA
1-800-264-0083 in Canada
Outside the USA and Canada, call your local Siemens representative.

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