Procedure Title:  **Tacrolimus – Abbott Architect**

Document Section:  **CHEMISTRY**  
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I. PURPOSE

The ARCHITECT Tacrolimus assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of tacrolimus in human whole blood on the ARCHITECT i System. The ARCHITECT Tacrolimus assay is to be used as an aid in the management of liver and kidney allograft patients receiving tacrolimus therapy.

SUMMARY AND EXPLANATION OF TEST

Tacrolimus is an immunosuppressive drug discovered in 1984 by the Fujisawa Pharmaceutical Co., Ltd. It has been shown to be effective for the treatment of organ rejection following transplantation. The results of clinical trials with liver and kidney have been published. Clinical studies are continuing for a variety of indications.

Tacrolimus binds to a family of proteins termed FK506 (tacrolimus) binding proteins (FKBPs). The formation of a larger pentameric complex comprised of FKBP, tacrolimus, calmodulin and calcineurins A and B results in the inhibition of the phosphatase activity of calcineurin. The action of transcription factors requiring dephosphorylation for transport to the cell nucleus are thus inhibited leading to blockage of T-cell proliferation and function.

Tacrolimus may be administered IV or orally. Absorption from the gastrointestinal tract is variable and irregular. Pharmacokinetic studies with tacrolimus have shown that there are large inter- and intra-individual differences in its kinetics in organ transplant patients.

Pharmacokinetic studies have also indicated that whole blood rather than plasma may serve as the more appropriate medium to describe the pharmacokinetic characteristics of tacrolimus. Tacrolimus is bound to proteins, mainly albumin and alpha-1-acid glycoprotein, and is highly bound to erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors such as hematocrit, temperature of separation of plasma, drug concentration, and plasma protein concentration. In a U.S. study, the ratio of whole blood concentration to plasma concentration ranged from 12 to 67 (mean 35).

Tacrolimus is extensively metabolized in the liver and small intestine microsomes utilizing the cytochrome P-450 enzymes. Nine different metabolites of tacrolimus have been identified; several of the metabolites have been found and tested in whole blood.

The use of tacrolimus is associated with serious toxic side effects, primarily nephrotoxicity. At the present time it is not clear whether the nephrotoxicity of tacrolimus is the result of parent drug, metabolites, or a combination of both. Other adverse side effects include neurotoxicity, hypertension, insomnia, and nausea.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Tacrolimus assay is a delayed one-step immunoassay for the quantitative determination of tacrolimus in human whole blood using CMIA technology with flexible assay protocols, referred to as Chemiflex.

Prior to the initiation of the automated ARCHITECT sequence, a manual pretreatment step is performed in which the whole blood sample is extracted with a precipitation reagent and centrifuged. The supernatant is decanted into a Transplant Pretreatment Tube, which is placed onto the ARCHITECT i System.

Sample, assay diluent, and anti-tacrolimus coated paramagnetic microparticles are combined to create a reaction mixture. Tacrolimus present in the sample binds to the anti-tacrolimus coated microparticles. After a delay, tacrolimus acridinium-labeled conjugate is added to the reaction mixture. The tacrolimus on the acridinium-labeled conjugate competes for the available binding sites on the microparticles. Following an incubation, the microparticles are washed, and pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs).
An indirect relationship exists between the amount of tacrolimus in the sample and the RLUs detected by the ARCHITECT i System optics.

For additional information on system and assay technology refer to the ARCHITECT System Operations Manual, Section 3.

II. POLICY/SCOPE

This procedure is intended for use by licensed CLS staff in the China Basin Chemistry section.

III. TEST AVAILABILITY

Test performed daily. Samples received in the lab by 12 noon will be resulted the same day before 1600.

IV. SPECIMEN REQUIREMENTS

SPECIMEN TYPES

1. Only human whole blood specimens collected in EDTA tubes may be used with the ARCHITECT Tacrolimus assay.

2. It is recommended that specimens be labeled with both the time of collection as well as the last drug administration.

3. Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient samples.

4. The ARCHITECT i System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen type is used in the ARCHITECT Tacrolimus assay.

STORAGE

1. Specimens collected in EDTA tubes may be stored up to 7 days refrigerated at 2-8 degrees C. prior to being tested. If testing will be delayed more than 7 days, store frozen (-10 degrees C. or colder). Specimens must be mixed thoroughly after thawing to ensure consistency of the results.

2. Avoid multiple freeze/thaw cycles.

3. Discard any remaining pretreated samples after testing is complete. ARCHITECT Cyclosporine tests cannot be reordered. A retest requires that the Manual Pretreatment Procedure in the PROCEDURE section be repeated.

V. EQUIPMENT, REAGENTS AND SUPPLIES

TEST INSTRUMENT: Abbott ARCHITECT System

MATERIALS PROVIDED

1. 1L77 ARCHITECT Tacrolimus Reagent Kit

2. 1L77-55 ARCHITECT Tacrolimus Whole Blood Precipitation Reagent

3. 9527-40 XSYSTEMS CENTRIFUGE TUBES

4. 1P06-01 Transplant Pretreatment Tubes

MATERIALS REQUIRED BUT NOT PROVIDED
1. ARCHITECT i System
2. 3K50 ARCHITECT i ASSAY CD-ROM - US - Addition A
3. 3K52 ARCHITECT i ASSAY CD-ROM - WW (excluding US) - Addition A
4. 1L77-01 ARCHITECT Tacrolimus Calibrators
6. Vortex Mixer
7. 9527-26 XSYSTEMS CENTRIFUGE
8. ARCHITECT i PRE-TRIGGER SOLUTION
9. ARCHITECT i TRIGGER SOLUTION
10. ARCHITECT i WASH BUFFER
11. ARCHITECT i REACTION VESSELS
12. ARCHITECT i SEPTUM
13. ARCHITECT i REPLACEMENT CAPS
14. Precision Micropipettes
15. Pipette tips
16. 9528-02 XSYSTEMS PRECISION DISPENSER, or equivalent
17. 2.5 mL Combitips, or equivalent, for PRECISION DISPENSER

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

REAGENTS

REAGENT KIT, 100 TESTS/500 TESTS

NOTE

Some kit sizes are not available in all countries or for use on all ARCHITECT i Systems. Please contact your local distributor.

ARCHITECT TACROLIMUS REAGENT KIT (1L77)

1. MICROPARTICLES: 1 Bottle (6.6 mL per 100 test bottle/27.0 mL per 500 test bottle) Anti-tacrolimus (mouse, monoclonal) coated microparticles in EDTA buffer with protein (bovine) stabilizer. Minimum Concentration: 0.09% solids. Preservatives: sodium azide and ProClin 950.

2. CONJUGATE: 1 Bottle (7.8 mL per 100 test bottle/16.0 mL per 500 test bottle) Tacrolimus acridinium-labeled conjugate in citrate buffer with protein (bovine) stabilizer. Minimum Concentration: 5.0 ng/mL. Preservative: ProClin 300.

3. ASSAY DILUENT: 1 Bottle (8.9 mL pr 100 test bottle/45.8 mL per 500 test bottle) Assay Diluent containing MES buffer and sodium chloride. Preservatives: ProClin 950 and ProClin 300.

OTHER REAGENTS
ARCHITECT i PRE-TRIGGER SOLUTION

PRE-TRIGGER SOLUTION: Pre-trigger solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT i TRIGGER SOLUTION

TRIGGER SOLUTION: Trigger solution containing 0.35 N sodium hydroxide.

ARCHITECT i WASH BUFFER


STORAGE INSTRUCTIONS

1. The ARCHITECT Tacrolimus Reagent Kit must be stored at 2-8 degrees C. in an upright position and may be used immediately after removal from 2-8 degrees C. storage.

2. When stored and handled as directed, reagents are stable until the expiration date.

3. The ARCHITECT Tacrolimus Reagent Kit may be stored on board the ARCHITECT i System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. Re-calibration may be required to obtain maximum onboard reagent stability. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

4. Reagents may be stored on or off the ARCHITECT i System. If reagents are removed from the system, store them at 2-8 degrees C. (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded. After reagents are removed from the system, initiate a reagent scan to update the onboard stability timer.

VI. WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use.

SAFETY PRECAUTIONS

CAUTION

This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

1. The microparticles, conjugate, and assay diluent contain methylisothiazolones which are components of ProClin and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

   R43  May cause sensitization by skin contact.
   S24  Avoid contact with skin.
   S35  This material and its container must be disposed of in a safe way.
   S37  Wear suitable gloves.
   S46  If swallowed, seek medical advice immediately and show this container or label.
2. For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

3. For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

HANDLING PRECAUTIONS

1. Do not use reagent kits beyond the expiration date.

2. Do not pool reagents within a kit or between reagent kits.

3. Before loading the ARCHITECT Tacrolimus Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the PROCEDURE, Assay Procedure section of this package insert.

4. Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.

5. To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
   a. Once a septum has been placed on the reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
   b. Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.

5. For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

VII. CALIBRATION/ CALIBRATION VERIFICATION

CALIBRATION

1. To perform an ARCHITECT Tacrolimus calibration, test calibrators A, B, C, D, E, and F in replicates of two. Only one pretreated sample of each ARCHITECT Tacrolimus Calibrator is required to perform a calibration on the ARCHITECT i System. This provides adequate volume to run each calibrator in duplicate. A single sample of each Tacrolimus control must be tested to evaluate the assay calibration. Ensure that assay control values are within established ranges.

2. Calibration Range: 0.0 - 30.0 ng/mL.

3. Once an ARCHITECT Tacrolimus calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
   a. A reagent kit with a new lot number is used.
   b. Controls are out of range.

3. For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

VIII. QUALITY CONTROL

BioRad Lyphochek Whole Blood Immunosuppressant controls Levels 1 and 4.

Reconstitution instructions.
Using a volumetric pipet, reconstitute each vial with 2.0 mL of deionized water. Replace the stopper.
and allow the control to stand for at least 60 minutes, swirling occasionally. Before sampling, gently swirl the contents until homogeneous with no visible signs of particulate. Stable for 14 days at 2-8°C

**Storage and stability.**
This product is stable until the expiration date when stored at 2-8°C. Once the control is reconstituted, all analytes will be stable for 14 days when stored at 2-8°C.

BioRad Immunosuppressant controls levels 1 and 4 are run each day of testing

### IX. PROCEDURE

**ASSAY PROCEDURE**

1. Before loading the ARCHITECT Tacrolimus Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.

   a. Invert the microparticle bottle 30 times by hand.

   b. Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue inverting the bottle until the microparticles have been completely resuspended.

   c. If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.

   d. Once the microparticles have been resuspended, place a septum on the bottle. For instructions on placing septums on bottles refer to the Handling Precautions section of this package insert.

2. Load the ARCHITECT Tacrolimus Reagent Kit on the ARCHITECT i System.

   a. Verify that all necessary reagents are present.

   b. Ensure that septums are present on all reagent bottles.

3. Order calibration, if necessary.

   For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.

4. Order tests.

   For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.

5. No more than 3 replicates may be sampled from the same Transplant Pretreatment Tube.

   a. All pretreated samples (specimens, calibrators or controls) must be tested within 30 minutes of being decanted into the Transplant Pretreatment Tubes and placed on the ARCHITECT i System.

   b. All ARCHITECT Tacrolimus samples must be priority loaded. Priority loading of samples prevents evaporation that will impact the assay results. No more than 100 Tacrolimus samples may be loaded onto the i2000 system at the same time. For information on priority loading of samples, refer to the ARCHITECT Systems Operation Manual, Section 5.

   c. With the Transplant Pretreatment Tube, use the sample gauge to ensure sufficient patient specimen is present for the ARCHITECT Tacrolimus assay.
6. Prepare calibrators and controls.

   Refer to the Manual Pretreatment Procedure in the PROCEDURE section.

7. Load pretreated samples.

   For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5

8. Press RUN.

9. For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.

10. For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. When a laboratory requires more frequent maintenance, follow those procedures.

SPECIMEN DILUTION PROCEDURES

Dilutions are not performed. Results above 30.0 ug/L are reported as >30.0

MANUAL PRETREATMENT

The ARCHITECT Tacrolimus assay requires a manual pretreatment step for all whole blood patient specimens, ARCHITECT Tacrolimus Calibrators and controls.

Use only ARCHITECT Tacrolimus Whole Blood Precipitation Reagent (1L77-55).

Once the Manual Pretreatment Procedure has been initiated, all steps must be completed in immediate succession.

WARNING:

Only Transplant Pretreatment Tubes (LN 1P06-01) are acceptable when pretreating tacrolimus samples for use on the ARCHITECT i System. Reliability of other ARCHITECT assay results may be affected if the Transplant Pretreatment Tubes are not used with the ARCHITECT Tacrolimus assay.

WARNING:

All pretreated samples (specimens, calibrators or controls) must be treated within 30 minutes of being decanted into the Transplant Pretreatment Tubes and placed on the ARCHITECT i System. All ARCHITECT Tacrolimus samples must be priority loaded. Priority loading of samples prevents evaporation that will impact the assay results. No more than 100 Tacrolimus samples may be loaded onto the i2000 system at the same time. For information on priority loading of samples, refer to the ARCHITECT Systems Operation Manual, Section 5.

MANUAL PRETREATMENT PROCEDURE

1. Mix each sample (specimen, calibrator, or control) thoroughly by slow inversion of the container 5-10 times. Visual inspection is recommended to assure the specimen is adequately mixed.

2. Precision pipette 200 uL of each sample into a labeled XSYSTEMS Centrifuge Tube immediately after mixing. Use a different tube for each sample.

NOTE:
A new pipette tip must be used each time 200 μL is aspirated. Do not wipe pipette tip. Do not over aspirate. Do not reuse pipette tips between replicates.

3a. Set a Precision Dispenser (Repeater Pipette) to dispense 200 μL. Fill the dispenser with a sufficient volume of the ARCHITECT Tacrolimus Whole Blood Precipitation Reagent from the blue-labeled bottle. NOTE: Do not process/dispense more than 4 tubes at a time.

Purge air bubbles from the dispenser by dispensing a small amount of the precipitation reagent into a suitable waste container.

NOTE:
The ARCHITECT Tacrolimus Whole Blood Precipitation Reagent is highly volatile. Keep it tightly closed when not in use to prevent evaporation.

3b. Add 200 μL of ARCHITECT Tacrolimus Whole Blood Precipitation Reagent to the contents of the first centrifuge tube (no more than 4 tubes at a time) with the end of the dispensing syringe tip touching the wall of the centrifuge tube.

3c. Cap the tube(s) and vortex immediately. Vortex up to 4 tubes at a time maximum.

3d. Vortex vigorously for 5-10 seconds. Use the maximum vortex setting.

WARNING:
Failure to vortex each tube immediately after addition of the ARCHITECT Tacrolimus Whole Blood Precipitation Reagent will lead to erroneous assay results.

NOTE:
Visual inspection is required to ensure that the mixture of the sample with the precipitation reagent is uniform, smooth and homogeneous.

No unmixed portion of the mixture should be present at the bottom of the tube. If unmixed sample remains, dislodge it by inverting the tube and tapping the bottom, then re-vortex the sample. This is an indication that the initial vortexing process was inadequate. Immediate vortexing minimizes the time available for aggregate formation. Not all vortex mixers may provide adequate mixing.

Repeat the "add, cap and vortex" process for each subsequent sample. For each tube, use a consistent vortexing time and complete the "add, cap and vortex" process before proceeding to the next tube. Do not dispense the ARCHITECT Tacrolimus Whole Blood Precipitation Reagent into all the tubes at once. Each individual tube must be capped and vortexed immediately after addition of the ARCHITECT Tacrolimus Whole Blood Precipitation Reagent before adding precipitation reagent to the subsequent tubes.

4. Load each tube into an XSYSTEMS Centrifuge taking care to balance the rotor. Centrifuge the tubes for 4 minutes at 13,000 rpm.

5. Remove each tube from the centrifuge and inspect for the presence of a well-formed pellet and clear supernatant.

6. Uncap each tube and decant (pour off) the supernatant into a labeled Transplant Pretreatment Tube.

WARNING:
Do not disturb the pellet. Do not pipette the supernatant as this will help ensure that the pellet is not disturbed.

NOTE:
Use a different Transplant Pretreatment Tube for each sample.

WARNING:

Only Transplant Pretreatment Tubes (LN 1P06-01) are acceptable when pretreating tacrolimus samples for use on the ARCHITECT i System. Reliability of other ARCHITECT assay results may be affected if the Transplant Pretreatment Tubes are not used with the ARCHITECT Tacrolimus assay.

WARNING:

All pretreated samples (specimens, calibrators or controls) must be tested within 30 minutes of being decanted into the Transplant Pretreatment Tubes and placed on the ARCHITECT i System. All ARCHITECT Tacrolimus samples must be priority loaded. Priority loading of samples prevents evaporation that will impact the assay results. No more than 100 Tacrolimus samples may be loaded onto the i2000 system at the same time. For information on priority loading of samples, refer to the ARCHITECT Systems Operation Manual, Section 5.

7. Vortex the Transplant Pretreatment Tube for 5-10 seconds.

8. Transfer the Transplant Pretreatment Tube to the ARCHITECT sample carrier.

9. Visually inspect the samples for presence of bubbles. If present, use a wooden applicator stick to remove bubbles.

NOTE:

Place the Transplant Pretreatment Tube so it touches the bottom of the carrier.

Discard any remaining pretreated samples after testing is complete. ARCHITECT Tacrolimus tests cannot be reordered. A retest requires that the Manual Pretreatment Procedure be repeated.

X. EXPECTED VALUES

5.0 to 20.0 ug/L

CAUTION

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, co-administration 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. Therefore, individual tacrolimus values cannot be used as the sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made. Each user must establish his or her 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 reported that the therapeutic range of tacrolimus is not clearly defined, but target 12-hour trough whole blood concentrations are 5-20 ng/mL early post-transplant. Higher concentrations are associated with an increase incidence of adverse effects. Twenty-four hour trough concentrations are 33-50% less than the corresponding 12-hour trough levels.

XI. LIMITATIONS OF PROCEDURE

1. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
2. If the tacrolimus results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

3. The concentration of tacrolimus in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

4. Immunoassays are nonspecific and cross react with metabolites. When elimination of tacrolimus is impaired (e.g. during cholestasis), tacrolimus metabolites may accumulate. The immunoassay may overestimate the concentration of tacrolimus. In such cases, the use of a specific assay (e.g. Liquid Chromatography Mass Spectrometry/Mass Spectrometry [LC/MS/MS]) could be considered. Refer to the SPECIFICITY section below for estimates of cross-reactivity of ARCHITECT Tacrolimus to some metabolites of tacrolimus. Refer to the METHOD COMPARISON section below for representative data comparing patient results from the ARCHITECT Tacrolimus assay to the IMx Tacrolimus II assay and an LC/MS/MS method.

5. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

6. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Specimens containing HAMA may produce anomalous values when tested with assay kits (such as ARCHITECT Tacrolimus) that employ mouse monoclonal antibodies.

XII. SPECIFICITY/INTERFERENCES

The ARCHITECT Tacrolimus assay is designed to have a mean recovery of 100 +/- 10% in the presence of the pharmaceutical substances, potentially interfering endogenous substances and potentially interfering clinical conditions at the levels below.

A study based on guidance from the CLSI document EP7-A2 was performed for the ARCHITECT Tacrolimus assay.

POTENTIALLY INTERFERING PHARMACEUTICAL SUBSTANCES

Whole blood specimens with tacrolimus concentrations between 4.9 and 19.8 ng/mL were supplemented with the following potentially interfering pharmaceutical substances. The average recovery observed during the study ranged from 95% to 104%.*

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Test Conc.</th>
<th>Test Compound</th>
<th>Test Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>20 mg/dL</td>
<td>Kanamycin B Sulfate</td>
<td>6 mg/dL</td>
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<tr>
<td>Acyclovir</td>
<td>3.2 µg/mL</td>
<td>Ketoconazole</td>
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<tr>
<td>Allopurinol</td>
<td>5 mg/dL</td>
<td>Labetalol</td>
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<td>Amikacin*2H₂O</td>
<td>15 mg/dL</td>
<td>Lidocaine</td>
<td>6 mg/dL</td>
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<td>Amphotericin B</td>
<td>5.8 µg/mL</td>
<td>Lovastatin</td>
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<tr>
<td>Apresoline</td>
<td>100 µg/mL</td>
<td>Minoxidil</td>
<td>60 µg/mL</td>
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<tr>
<td>Azathioprine</td>
<td>1 mg/dL</td>
<td>Micophenolic Acid</td>
<td>500 µg/mL</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>8 µg/mL</td>
<td>Micophenolic Acid Glucuronide</td>
<td>1800 µg/mL</td>
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<tr>
<td>Carbamazepine</td>
<td>12 mg/dL</td>
<td>N-Acetyl-Procainamide</td>
<td>12 mg/dL</td>
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<tr>
<td>Cephalosporine</td>
<td>100 µg/mL</td>
<td>Nadolol</td>
<td>1.2 µg/mL</td>
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<tr>
<td>Chloramphenicol</td>
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<td>Nicardipine</td>
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<td>Chloroquine</td>
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<td>PenicillinG Na+</td>
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<td>Cimetidine</td>
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<td>Prednisolone</td>
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<tr>
<td>Cortisone</td>
<td>1.2 µg/mL</td>
<td>Prednisone</td>
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### Cyclosporine

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<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine</td>
<td>3200 ng/mL</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>80 ng/mL</td>
</tr>
<tr>
<td>Digoxin</td>
<td>4.8 ng/mL</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>60 µg/mL</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>3 mg/dL</td>
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<tr>
<td>Erythromycin</td>
<td>20 mg/dL</td>
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<tr>
<td>Flucytosine</td>
<td>40 µg/mL</td>
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<tr>
<td>Furosemide</td>
<td>2 mg/dL</td>
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<td>Ganciclovir</td>
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<td>Gemfibrozil</td>
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<td>Gentamicin</td>
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<td>Itraconazole</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Kanamycin A Sulfate</td>
<td>6 mg/dL</td>
</tr>
<tr>
<td>Primidone</td>
<td>600 µg/mL</td>
</tr>
<tr>
<td>Probenecid</td>
<td>10 mg/dL</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.5 mg/dL</td>
</tr>
<tr>
<td>Quinidine</td>
<td>5 mg/dL</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>60 ng/mL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>800 mg/dL</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>≤ 25%, ≥ 55%</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>40 mg/dL</td>
</tr>
<tr>
<td>Total Protein</td>
<td>3 g/dL</td>
</tr>
<tr>
<td>Total Protein</td>
<td>12 g/dL</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>500 mg/dL</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>20 mg/dL</td>
</tr>
</tbody>
</table>

---

**POTENTIALLY INTERFERING ENDOGENOUS SUBSTANCES**

Whole blood specimens with tacrolimus concentrations between 5.5 and 18.0 ng/mL were supplemented with the following potentially interfering endogenous substances. The average recovery observed during the study ranged from 96% to 105%.*

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>800 mg/dL</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>≤ 25%, ≥ 55%</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>40 mg/dL</td>
</tr>
<tr>
<td>Total Protein</td>
<td>3 g/dL</td>
</tr>
<tr>
<td>Total Protein</td>
<td>12 g/dL</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>500 mg/dL</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>20 mg/dL</td>
</tr>
</tbody>
</table>

---

XIII. **SPECIFIC PERFORMANCE CHARACTERISTICS**

**Precision**

The ARCHITECT Tacrolimus assay is designed to have precision of ≤ 10% total CV. A study was performed with the ARCHITECT Tacrolimus assay based on guidance from the Clinical and Laboratory Standards Institute, (CLSI, formerly NCCLS) document EP5-A2.30 Abbott Immunosuppressant-MCC (levels 1, 2 and 3) and five whole blood panels were assayed, using two lots of reagents, on two instruments, in replicates of two at two separate times per day for 20 days. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized in the following table.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Instrument</th>
<th>Reagent Lot</th>
<th>n</th>
<th>Mean (ng/mL)</th>
<th>Within Run SD</th>
<th>%CV</th>
<th>Total SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>1</td>
<td>1, 2</td>
<td>80</td>
<td>3.0</td>
<td>0.1</td>
<td>3.7</td>
<td>0.1</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1, 2</td>
<td>80</td>
<td>2.9</td>
<td>0.2</td>
<td>5.8</td>
<td>0.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Level 2</td>
<td>1</td>
<td>1, 2</td>
<td>80</td>
<td>7.8</td>
<td>0.2</td>
<td>2.4</td>
<td>0.3</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1, 2</td>
<td>80</td>
<td>8.5</td>
<td>0.2</td>
<td>2.7</td>
<td>0.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Level 3</td>
<td>1</td>
<td>1, 2</td>
<td>80</td>
<td>14.5</td>
<td>0.4</td>
<td>2.5</td>
<td>0.5</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1, 2</td>
<td>80</td>
<td>15.7</td>
<td>0.5</td>
<td>2.9</td>
<td>0.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Panel 1</td>
<td>1</td>
<td>1, 2</td>
<td>80</td>
<td>5.5</td>
<td>0.2</td>
<td>3.6</td>
<td>0.2</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1, 2</td>
<td>80</td>
<td>5.9</td>
<td>0.2</td>
<td>4.0</td>
<td>0.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Panel 2</td>
<td>1</td>
<td>1, 2</td>
<td>80</td>
<td>14.0</td>
<td>0.5</td>
<td>3.5</td>
<td>0.6</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1, 2</td>
<td>80</td>
<td>15.3</td>
<td>0.6</td>
<td>4.1</td>
<td>0.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Panel 3</td>
<td>1</td>
<td>1, 2</td>
<td>80</td>
<td>4.8</td>
<td>0.2</td>
<td>4.4</td>
<td>0.2</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1, 2</td>
<td>80</td>
<td>4.9</td>
<td>0.2</td>
<td>5.0</td>
<td>0.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Panel 4</td>
<td>1</td>
<td>1, 2</td>
<td>80</td>
<td>10.1</td>
<td>0.2</td>
<td>2.4</td>
<td>0.4</td>
<td>4.4</td>
</tr>
</tbody>
</table>
Specificity

A study was performed with the ARCHITECT Tacrolimus assay based on guidance from CLSI document EP7-A2.31. Aliquots of whole blood specimens were supplemented with tacrolimus, targeting values ranging from 5 to 22 ng/mL. These specimens were spiked with cross-reactant solutions. Data from this study are summarized in the following table.* Tacrolimus metabolites that have been detected in human blood were tested in the ARCHITECT Tacrolimus assay. Physiological concentrations of the tacrolimus metabolites in whole blood and the clinical significance of the tacrolimus metabolites have not been defined. Purified tacrolimus metabolites are not commercially available for cross-reactivity testing. Tacrolimus metabolites were prepared in vitro by incubating tacrolimus with liver microsomes prepared from phenobarbital-treated rats in the presence of NADPH generating system under aerobic condition or bioconverted by incubating tacrolimus with an actinomycete. Oxidative metabolites formed in the reaction medium were isolated and identified. Purified samples were analyzed by HPLC, mass spectrometry, and NMR spectroscopy.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Amount Added (ng/mL)</th>
<th>Mean Excess Concentration Detected (ng/mL, n=5)</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-I (13-O-demethyltacrolimus)</td>
<td>10</td>
<td>0.8</td>
<td>8</td>
</tr>
<tr>
<td>M-II (31-O-demethyltacrolimus)</td>
<td>10</td>
<td>9.4</td>
<td>94</td>
</tr>
<tr>
<td>M-III (15-O-demethyltacrolimus)</td>
<td>10</td>
<td>4.5</td>
<td>45</td>
</tr>
<tr>
<td>M-IV(12-hydroxytacrolimus)</td>
<td>10</td>
<td>0.8</td>
<td>9</td>
</tr>
</tbody>
</table>

Sensitivity

The ARCHITECT Tacrolimus assay is designed to have a limit of detection of \( \leq 1.5 \) ng/mL. The limit of detection of the ARCHITECT Tacrolimus assay, defined as the concentration at two standard deviations above the ARCHITECT Tacrolimus Calibrator A (0 ng/mL), was calculated to be 1.5 ng/mL* at the 95% level of confidence (based upon one study with n=24 runs, 10 replicates calibrator A and 4 replicates calibrator B per run). Representative data; results in individual laboratories may vary from these data.

XIV. ALTERNATE METHODS

The Architect i1000 analyzer is the backup method if the Architect i2000 is not available for testing.

XV. REFERENCES

ABBOTT ARCHITECT Cyclosporine package insert
Abbott Laboratories
Diagnostics Division
Abbott Park, IL  60064
PN 301-512 8/08
Revision 09/08