This package insert must be read carefully prior to product use. Package insert instructions must be followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

- **LOT**: Lot Number
- **CONTROL NO.**: Control Number
- **ASSAY CD-ROM**: Assay CD-ROM
- **REACTION VESSELS**: Reaction Vessels
- **SEPTUM**: Septum
- **REPLACEMENT CAPS**: Replacement Caps
- **CENTRIFUGE TUBES**: Centrifuge Tubes
- **CENTRIFUGE**: Centrifuge
- **PRECISION DISPENSER**: Precision Dispenser
- **STERILE**: Sterile, method of sterilization using irradiation
- **Do not re-use**: Do not re-use

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.
NAME
ARCHITECT Tacrolimus

INTENDED USE
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 allograft patients receiving tacrolimus therapy 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 -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 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. Other adverse side effects include neurotoxicity, a combination of both. Other adverse side effects include myelosuppression, hypertension, insomnia, and nausea.

BIological PRINCIPLES OF THE PROCEDURE
The ARCHITECT Tacrolimus assay is a chemiluminescent microparticle immunoassay for the quantitative determination of tacrolimus in human whole blood using CMIA technology with flexible assay protocols, referred to as Chemiflex. The ARCHITECT Tacrolimus assay is based on the reaction of anti-tacrolimus coated microparticles with drug and drug conjugate present 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.

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)

- **INTRANALYSIS**
  - 1 Bottle (7.8 mL per 100 test bottle/16.0 mL per 500 test bottle) Anti-tacrolimus (mouse, monospecific) coated microparticles in assay diluent buffer with protein (bovine) stabilizer. Minimum Concentration: 0.09% solids. Preservatives: sodium azide and ProClin 940.

- **COMPONENT**
  - 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.

- **ASSAY BUFFER**
  - 1 Bottle (4.0 mL per 100 test bottle/8.0 mL per 500 test bottle) Assay Diluent containing MES buffer and sodium chloride. Preservatives: ProClin 300 and ProClin 500.

Other Reagents

ARCHITECT i Pre-Trigger Solution

- **PRETRIGGER 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

- **WASH BUFFER**
  - 15 mL buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

**WP**

**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. Safety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

- The microparticles, conjugates, and assay diluent contain methylisothiazolones, which are components of ProClin, and are classified as irritants under applicable European Community (EC) Directives as irritant (I). 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.**

For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

This product contains sodium azide; for a specific listing, refer to the REAGENTS section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

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

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not pool reagents within a kit or between reagent kits.
- 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.
• 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.

• To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.

• Once a septum has been placed on the reagent bottle, do not insert the bottle as this will result in reagent leakage and may compromise assay results.

• Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.

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

Stability

Results and Stability

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

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

• The ARCHITECT Tacrolimus Reagent Kit must be stored on board the ARCHITECT 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.

• Reagents may be stored on or off the ARCHITECT System. If reagents are removed from the system, store them at 2-8°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 bags 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. For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

• When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

• The ARCHITECT Tacrolimus assay file must be installed on the ARCHITECT System from the ARCHITECT 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.

• For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

• For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
Once the Manual Pretreatment Procedure (1L77-55) has been initiated, all steps must be completed in immediate succession.

Attention:

• Use only ARCHITECT Tacrolimus Whole Blood Precipitation Reagent to the ARCHITECT System Operations Manual, Section 9.

For information on materials required for maintenance procedures, refer to the ARCHITECT Systems Operation Manual, Section 5.

Note:

Support Center or your Abbott Representative.

Note:

Warning: Only Transplant Pretreatment Tubes (LN 1P06-01) are acceptable when pretreating tacrolimus samples for use on the ARCHITECT 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 (specimen, calibrators or controls) must be tested within 30 minutes of being decanted into the Transplant Pretreatment Tubes and placed on the ARCHITECT 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 into the XSYSTEMS 2000 system at the same time.

Note: An ARCHITECT Tacrolimus Sample Pretreatment Guide outlining the pretreatment steps is also available from the ARCHITECT Customer Support Center or your Abbott Representative.

Manual Pretreatment Procedure

The ARCHITECT Tacrolimus assay requires a manual pretreatment step for all whole blood patient specimens, ARCHITECT Tacrolimus Calibrators and Abbott Immunosuppressant-MCC or other 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.

Note: If specimen requires dilution, it must be diluted prior to the manual pretreatment step. Refer to the Specimen Dilution Procedures section of this package insert.

Warning: Only Transplant Pretreatment Tubes (LN 1P06-01) are acceptable when pretreating tacrolimus samples for use on the ARCHITECT 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 (specimen, calibrators or controls) must be tested within 30 minutes of being decanted into the Transplant Pretreatment Tubes and placed on the ARCHITECT 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 into the XSYSTEMS 2000 system at the same time.

For each tube, use a consistent vortexing time and complete the "add, cap and vortex" process before proceeding to the next tube. Do not disperse 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.

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.

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

Note: To prevent leaking, do not place a filled repeater pipette on the lab bench. 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 with the end of the dispensing syringe tip touching the wall of the centrifuge tube.

Warning: Each individual tube must be capped and vortexed immediately after addition of the ARCHITECT Tacrolimus Whole Blood Precipitation Reagent, before adding the precipitation reagent to subsequent tubes.

3c. Cap the first tube and vortex immediately.

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 sunned portion of the mixture should be present at the bottom of the tube. If sunned sample remains, dispose of 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 disperse 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. A balance tube can be added if necessary. Only an even number of tubes can be centrifuged at one time.

Centrifuge the tubes for 5-10 minutes.

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 the Transplant Pretreatment Tube, when the ARCHITECT i System is ready to accept samples.

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 System. Reliability of other ARCHITECT assay results may be affected if the Transplant Pretreatment Tubes are not used with the ARCHITECT Tacrolimus assay.

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.

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

Note: To prevent leaking, do not place a filled repeater pipette on the lab bench. 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 with the end of the dispensing syringe tip touching the wall of the centrifuge tube.

Warning: Each individual tube must be capped and vortexed immediately after addition of the ARCHITECT Tacrolimus Whole Blood Precipitation Reagent, before adding the precipitation reagent to subsequent tubes.

3c. Cap the first tube and vortex immediately.

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 sunned portion of the mixture should be present at the bottom of the tube. If sunned sample remains, dispose of 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 disperse 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. A balance tube can be added if necessary. Only an even number of tubes can be centrifuged at one time.

Centrifuge the tubes for 5-10 minutes.

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 the Transplant Pretreatment Tube, when the ARCHITECT i System is ready to accept samples.

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 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 loaded 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.

Prior to loading samples, ensure that the Transplant Pretreatment Tubes and samples are in the correct position on the system prior to the start of the assay.

1. Prior to loading the Reagent Kit, refer to the ARCHITECT Systems Operation Manual, Section 5.

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

3. Set up the Transplant Pretreatment Tube for 5 - 10 minutes.

4. 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.

5. Press the RUN button.

6. For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

7. Perform a check of the system to ensure that the Manual Dilution Procedure is applied. If the system is unable to perform the proper dilution, the appropriate dilution factor must be entered manually.

8. Once a specimen is loaded onto the system and the system is ready, the assay will begin. The assay will continue until the assay limit is reached or the specimen dilution limit is reached. If the specimen dilution limit is reached, the specimen will be discarded and the assay will be repeated.

9. When a laboratory requires more frequent maintenance, follow those procedures.

Assay Procedure

• 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. After the first time the microparticles have been loaded, no further mixing is required.

• Invert the microparticle bottle 30 times.

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

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

• 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.

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

• Verify that all necessary assay reagents are present.

• Ensure that reagents are present on all reagent bottles.

• Order calibration, if necessary.

• For information on ordering calibrators, refer to the ARCHITECT System Operations Manual, Section 5.

• Once the microparticles have been resuspended, the sample is ready to be loaded onto the system. For instructions on placing septums on bottles refer to the Handling Precautions section of this package insert.

• Load the ARCHITECT Tacrolimus Reagent Kit 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. No more than 100 Tacrolimus samples may be loaded onto the i2000 system at the same time. No more than 100 Tacrolimus samples may be loaded onto the i2000 system at the same time. 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 Operations Manual, Section 5.

• Prepare calibrators and controls.

• Refer to the Manual Procedure in the PROCEDURE section.

• Load pretreated samples.

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

• Press RUN.

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

• 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

Specimens with a tacrolimus concentration of > 30.0 ng/mL will be flagged as "> 30.0 ng/mL" and may be diluted with the Manual Dilution Procedure.

Manual dilutions should be performed as follows:

• The suggested dilution for the ARCHITECT Tacrolimus assay is 1:2.

• Specimen must be diluted before pretreatment.

• Add 150 μL of the patient specimen to 150 μL of ARCHITECT Tacrolimus Calibrator A, then proceed with the Manual Pretreatment Procedure in the PROCEDURE section.

• The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The result (before dilution factor is applied) should be greater than 3.0 ng/mL.

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

• 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.

• Calibration Range: 0.0 - 30.0 ng/mL

• Once an ARCHITECT Tacrolimus calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

• A reagent kit with a new lot number is used.

• Controls are out of range.

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

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT Tacrolimus assay is that a single sample of each control level be tested once every 24 hours each day of use. Commercial controls such as the Abbott Immunosuppressant-MCC are suitable for this purpose. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow those procedures.

Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated test results are invalid and must be repeated. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT Tacrolimus assay belongs to method group B.

ARCHITECT Tacrolimus Calibrators may be used when MasterCheck (4PLC, Y-weighted) data reduction method is not available. Refer to the ARCHITECT System Operations Manual, Appendix B.

RESULTS

The ARCHITECT Tacrolimus assay uses a 4 Parameter Logistic/Curve Fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.
**LIMITATIONS OF THE PROCEDURE**

- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- If the tacrolimus results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- 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.
- 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.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. 
- Panels or specimens containing HMA may produce anomalous values when tested with assay kits (such as ARCHITECT Tacrolimus) that employ mouse monoclonal antibodies.

**EXPECTED VALUES**

**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.

**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 EP2-A2. 

**CAUTION:** Abbott Immunoassayest-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 1. Specific Performance Characteristics

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Endogenous Concentration (ng/mL)</th>
<th>Measured Concentration (ng/mL)</th>
<th>Recovery (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.5</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.9</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9.3</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>15.2</td>
<td>16.1</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>18.8</td>
<td>18.6</td>
<td>99</td>
</tr>
</tbody>
</table>

**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.
Dilution Linearity

The ARCHITECT Tacrolimus assay is designed to have a mean recovery of 100 ± 10% of the expected results for diluted samples. A dilution linearity study was performed by diluting high concentration tacrolimus whole blood specimens with the ARCHITECT Tacrolimus Calibrator A. The concentration of tacrolimus was determined for each dilution of sample and the percent (%) recovery was calculated. Data from this study are summarized in the following table.*

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Dilution Factor</th>
<th>Observed Concentration (ng/mL)</th>
<th>Calculated Concentration (ng/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Undiluted</td>
<td>29.4</td>
<td>29.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:11</td>
<td>26.7</td>
<td>26.5</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>1:25</td>
<td>23.0</td>
<td>23.6</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1:4.3</td>
<td>20.7</td>
<td>23.6</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1:11</td>
<td>17.3</td>
<td>23.9</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>11.7</td>
<td>23.9</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>1:10</td>
<td>6.0</td>
<td>30.0</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>1:50</td>
<td>0.8</td>
<td>28.0</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>Undiluted</td>
<td>27.8</td>
<td>27.8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:11</td>
<td>25.6</td>
<td>28.4</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>1:25</td>
<td>23.3</td>
<td>29.1</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>1:4.3</td>
<td>20.1</td>
<td>28.7</td>
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</tr>
<tr>
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<td>1:11</td>
<td>17.3</td>
<td>25.9</td>
<td>108</td>
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<tr>
<td></td>
<td>1:5</td>
<td>11.9</td>
<td>25.9</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>1:10</td>
<td>5.8</td>
<td>29.0</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>1:50</td>
<td>0.8</td>
<td>29.0</td>
<td>104</td>
</tr>
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<td>3</td>
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<td>28.1</td>
<td>28.1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:11</td>
<td>25.3</td>
<td>28.1</td>
<td>100</td>
</tr>
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<td></td>
<td>1:25</td>
<td>22.9</td>
<td>28.5</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>1:4.3</td>
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</tr>
<tr>
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<td>17.2</td>
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</tr>
<tr>
<td></td>
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<td>11.7</td>
<td>23.3</td>
<td>104</td>
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<tr>
<td></td>
<td>1:10</td>
<td>5.9</td>
<td>23.5</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>1:50</td>
<td>0.8</td>
<td>28.0</td>
<td>100</td>
</tr>
</tbody>
</table>

* Calculated Concentration = Observed Concentration x Dilution Factor
% Recovery = Calculated Concentration / Undiluted Observed Concentration x 100

Recovery from these data

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Dilution Factor</th>
<th>Observed Concentration (ng/mL)</th>
<th>Calculated Concentration (ng/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Undiluted</td>
<td>29.4</td>
<td>29.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:11</td>
<td>26.7</td>
<td>26.5</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>1:25</td>
<td>23.0</td>
<td>23.6</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1:4.3</td>
<td>20.7</td>
<td>23.6</td>
<td>98</td>
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</tr>
<tr>
<td></td>
<td>1:5</td>
<td>11.7</td>
<td>23.9</td>
<td>99</td>
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<td></td>
<td>1:10</td>
<td>6.0</td>
<td>30.0</td>
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</tr>
<tr>
<td></td>
<td>1:50</td>
<td>0.8</td>
<td>28.0</td>
<td>95</td>
</tr>
</tbody>
</table>

Specificity

A study was performed with the ARCHITECT Tacrolimus assay based on guidance from CLSI document EPT-A2. Of allogeneic 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.*

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Amount Added (ng/mL, n=5)</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1 (15-O-demethyltacrolimus)</td>
<td>10 0.8 8</td>
<td></td>
</tr>
<tr>
<td>M-2 (13-O-demethyltacrolimus)</td>
<td>10 9 94</td>
<td></td>
</tr>
<tr>
<td>M-3 (15-O-demethyltacrolimus)</td>
<td>10 4.5 45</td>
<td></td>
</tr>
<tr>
<td>M-V (12-hydroxytacrolimus)</td>
<td>10 0.8 9</td>
<td></td>
</tr>
</tbody>
</table>

* Cross-reactivities as estimated by interference with the measurement of tacrolimus in whole blood specimens

**Representative data; results in individual laboratories may vary from these data.

Interference

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 EPT-A2 was performed for the ARCHITECT Tacrolimus assay.

Potentially Interfering Pharmacological 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%.

Functional Sensitivity

The ARCHITECT Tacrolimus assay is designed to have a functional sensitivity of ≤ 2 ng/mL.

A study was performed where whole blood specimens were spiked with tacrolimus to achieve approximate concentrations from 0.2 to 4.4 ng/mL. These were tested in replicates of ten, twice a day for five days using one reagent and calibrator lot for a total of 100 replicates per panel. The total % CVs were calculated and plotted against the mean concentration. A reciprocal curve was fitted through the data and the functional sensitivity value was calculated as the concentration corresponding to the 20% CV level of the fitted curve. At the upper 95% confidence limit, the lowest ARCHITECT Tacrolimus assay value exhibiting a 20% CV was calculated to be 0.9 ng/mL, which is below the reportable range of the ARCHITECT Tacrolimus assay.*

* Representative data; results in individual laboratories may vary from these data.
**Method Comparison**

The ARCHITECT Tacrolimus assay is designed to have a correlation coefficient of ≥ 0.90 for specimens between 2 – 30 ng/mL when compared to the LC/MS/MS method. Data from this study are summarized in the following table.*

<table>
<thead>
<tr>
<th>Specimen Range (ARCHITECT): 2.2 ng/mL to 14.8 ng/mL</th>
<th>Specimen Range (LC/MS/MS): 2.1 ng/mL to 15.9 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen Range (IMx): 2.1 ng/mL to 15.9 ng/mL</td>
<td>Specimen Range (LC/MS/MS): 1.78 ng/mL to 19.20 ng/mL</td>
</tr>
</tbody>
</table>

A bias analysis of the ARCHITECT Tacrolimus vs. IMx Tacrolimus II assay was performed on the same 124 human whole blood EDTA samples in the range of 2 to 30 ng/mL. The following representative data are provided to aid in understanding the differences between these two assays. The average ng/mL difference bias exhibited by ARCHITECT Tacrolimus vs. IMx Tacrolimus II assay in this study was -0.94 ng/mL. The 95% confidence interval of the ng/mL difference bias is -0.16 ng/mL to -0.71 ng/mL. Results of the study are summarized below.

A bias analysis of the ARCHITECT Tacrolimus vs. LC/MS/MS was performed on the same 125 human whole blood EDTA samples in the range of 2 to 30 ng/mL. The following representative data are provided to aid in understanding the differences between these two assays. The average ng/mL difference bias exhibited by ARCHITECT Tacrolimus vs. LC/MS/MS in this study was 0.51 ng/mL. The 95% confidence interval of the ng/mL difference bias is 0.02 ng/mL to 0.48 ng/mL. Results of the study are summarized below.

---

* Representative data; results in individual laboratories may vary from these data.

**Additional testing of the above samples was completed with LC/MS/MS where regression analysis was performed using the Passing-Bablok**

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**Test Compound** | **Test Conc.** | **Test Compound** | **Test Conc.**
---|---|---|---|
Lipidic Acid | 74 mg/dL | Phenylalanine | 10 mg/dL |
Cholesterol | 0.01 mg/dL | Propranolol | 25 μg/mL |
Creatinine | 0.09 mg/dL | Prednisolone | 100 μg/mL |
Uric Acid | 12 μg/mL | Prednisone | 100 μg/mL |
Lactate | 3200 mg/dL | Prinamide | 10 mg/dL |
Diphenhydramine | 60 mg/dL | Propranolol | 0.5 mg/dL |
Diphenhydramine | 3 mg/dL | Quinidine | 5 mg/dL |
Epinephrine | 20 mg/dL | Ramipril | 20 mg/dL |
Fluconazole | 30 mg/dL | Rifampin | 5 mg/dL |
Fluoxetine | 40 mg/dL | Sinusine | 60 mg/dL |
Fosumegide | 2 mg/dL | Specynomycin | 100 μg/mL |
Ganciclovir | 1000 μg/mL | Ticlopidine | 150 μg/mL |
Gentamicin | 500 μg/mL | Toremycin | 2 mg/dL |
Gentamicin | 12 mg/L | Trimethoprin | 40 μg/mL |
Hydrocortisone | 12 μg/mL | Valproic Acid | 50 mg/dL |
Inosine | 50 mg/mL | Vancomycin | 6 mg/dL |
Kanamycin A Sulfate | 6 mg/dL | Verapamil | 10 μg/mL |
*Cholesterol | 500 mg/dL | Valproic Acid | 50 mg/dL |
*Total Protein | 12 g/dL | Valproic Acid | 50 mg/dL |
*Hematocrit | ≤ 25%, ≥ 55% | Vancomycin | 6 mg/dL |
*Triglycerides | 800 mg/dL | Verapamil | 10 μg/mL |
*Potential 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 98% to 105%.*

---

**Related Clinical Conditions**

The ARCHITECT Tacrolimus assay was evaluated using specimens with HAMA and RF to further assess the clinical specificity. Five specimens positive for HAMA and five specimens positive for RF were evaluated for % recovery with tacrolimus spiked at two concentrations into each specimen between 7.1 and 20.0 ng/mL. Mean percent recovery results are summarized in the following table.*

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>Number of Specimens</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAMA</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>RF</td>
<td>10</td>
<td>90%</td>
</tr>
</tbody>
</table>

---

**Method Comparison**

The ARCHITECT Tacrolimus assay is designed to have a correlation coefficient of ≥ 0.90 for specimens between 2 – 30 ng/mL, when compared to the Imx Tacrolimus II assay. A study was performed to compare the ARCHITECT Tacrolimus assay to the Imx Tacrolimus II assay using human whole blood EDTA specimens from liver and kidney transplant patients receiving tacrolimus therapy. Regression analysis was performed using the Passing-Bablok** method. Data from this study are summarized in the following table.*

<table>
<thead>
<tr>
<th>Observations</th>
<th>Intercept (95% CI)</th>
<th>Slope (95% CI)</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>124</td>
<td>0.37 (0.05 to 0.68)</td>
<td>0.81 (0.46 to 0.88)</td>
<td>0.90</td>
</tr>
</tbody>
</table>
BIBLIOGRAPHY


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