# Sirolimus – Abbott Architect

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**Document Section:** CHEMISTRY  
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I. PURPOSE

The ARCHITECT Sirolimus assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of sirolimus in human whole blood on the ARCHITECT i System. The ARCHITECT Sirolimus assay is to be used as an aid in the management of renal transplant patients receiving sirolimus therapy.

SUMMARY AND EXPLANATION OF TEST

Sirolimus (Rapamune, rapamycin, Wyeth Pharmaceuticals, Collegeville, PA) is an immunosuppressive drug for renal transplant immunosuppressive therapy. The safety and efficacy of sirolimus in helping prevent tissue rejection was initially demonstrated in two multicenter trials (Trials 301 and 302) involving postrenal transplant patients receiving full-dose cyclosporine and corticosteroids. The data indicated beneficial prophylaxis against acute rejection from sirolimus therapy in conjunction with cyclosporine and corticosteroids. Subsequently, the safety and efficacy of sirolimus as a maintenance regimen following cyclosporine withdrawal was assessed. In this study, clinical outcomes of patients withdrawn from cyclosporine and maintained on sirolimus and corticosteroids compared favorably to patients continuing on the triple-drug immunosuppressive regimen. Because of potential toxic effects associated with high trough levels of sirolimus, therapeutic drug monitoring of sirolimus immunosuppressive therapy has been recommended. Sirolimus is a macrocyclic lactone fermentation product of Streptomyces hygroscopicus, first discovered in the soil of Rapa Nui (Easter Island). Sirolimus exhibits a synergistic action with calcineurin inhibitors (e.g., cyclosporine), although it operates with a different mechanism. Sirolimus binds to the immunophilin FK-binding protein 12, and the resulting complex binds to a specific cell-cycle regulatory protein mTOR (mammalian target of rapamycin) and inhibits its activation. The inhibition of mTOR results in suppression of cytokine-driven T-lymphocyte proliferation, inhibiting the progression from G1 to the S phase of the cell cycle. Pharmacokinetic studies indicate that sirolimus is primarily sequestered in erythrocytes, and that the appropriate sample medium with which to monitor sirolimus is whole blood.

The bioavailability of sirolimus was estimated to be 14% after the administration of sirolimus oral solution and the mean bioavailability of sirolimus after administration of the tablet is about 27% higher relative to the oral solution. Ascending dose studies (range 0.5 - 6.5 mg/m to the 2nd power/12 hrs) showed peak whole-blood concentrations of 10 - 210 ng/mL and mean time to peak concentration of 1.4 +/- 1.2 (range 0.7 - 3) hours. A good correlation (r to the 2nd power = 0.85) of trough concentration to area under the concentration time curve (AUC) was found; therefore trough concentration measurement provides a useful index of total drug exposure during the dosing interval.

Among 30 stable renal allograft recipients who received a 14-day course of sirolimus concomitantly with cyclosporine and corticosteroids, there was a 4.5 fold difference in apparent mean drug clearance of 208 +/- 95 mL/h/kg and a terminal half-life of 62 +/- 16 hours. Because of the long half-life, trough levels should be monitored no less than 5 - 7 days after a dosage change. Once a day dosing is recommended in adult renal transplant patients. A loading dose (3 times the maintenance dose) can be used to achieve near steady-state blood concentrations rapidly. Variations in apparent drug clearance and oral bioavailability result in a wide range of sirolimus trough values among patients receiving identical doses.

Sirolimus is a substrate for the cytochrome P450 IIIA4 (CYP3A4 isozyme) and p-glycoprotein transporter and is extensively metabolized by O-demethylation and/or hydroxylation. Therefore, drugs that are known inducers or inhibitors of these two pathways have the ability to dramatically decrease or increase sirolimus whole blood concentrations, respectively. The immunosuppressive activity of sirolimus metabolites is thought to be no more than about 10% relative to the parent drug. A preliminary study using HPLC/MS/MS suggests that the steady-state profile of sirolimus metabolites is consistent between patients. For a small number of patients tested (n=2) the profile was also shown to be consistent over time. Consistency in metabolite profiles should contribute to a good correlation between methods that are specific for parent drug and the methods that detect both parent drug and its metabolites.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE
The ARCHITECT Sirolimus assay is a delayed one-step immunoassay for the quantitative determination of sirolimus 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, heated, and centrifuged. The supernatant is decanted into a Transplant Pretreatment Tube, which is placed onto the ARCHITECT i System.

Sample, assay diluent and anti-sirolimus coated paramagnetic microparticles are combined to create a reaction mixture. Sirolimus present in the sample binds to the anti-sirolimus coated microparticles. After a delay, sirolimus acridinium-labeled conjugate is added to the reaction mixture. The sirolimus acridinium-labeled conjugate competes for the available binding sites on the anti-sirolimus coated paramagnetic 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 sirolimus 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 Sirolimus assay.

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

3. 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 Sirolimus assay.

STORAGE

1. Specimens collected in EDTA tubes may be stored up to 7 days at 2-8 degrees C.

2. ARCHITECT Sirolimus values may shift slightly during 2-8 degrees C. storage or after 1 freeze/thaw cycle. Grand mean recovery of the refrigerated samples after 7 days storage was 96% and grand mean recovery of the frozen samples after 1 freeze/thaw cycle was 104%. However, some individual values were > +/- 20% of the original value. The results below were obtained in a stability study using fresh clinical specimens tested after either 7 days storage at 2-8 degrees C. or after 1 freeze/thaw cycle shown as a concentration change from Day 0.*

Initial Specimen Conc. Measured on Day 0:  5.3 to 7.3 ng/mL
Average Change in Conc. after 7 days at 2-8 degrees C.:  0.1 ng/mL
Range:  -0.4 to 1.1 ng/mL
n:  4
Average Change in Conc. after 1 freeze/thaw: 0.4 ng/mL  
Range: -0.9 to 1.1 ng/mL  
n: 12

Initial Specimen Conc. Measured on Day 0: 12.1 to 15.9 ng/mL  
Average Change in Conc. after 7 days at 2-8 degrees C.: -1.1 ng/mL  
Range: -4.4 to 0.8 ng/mL  
n: 5

Average Change in Conc. after 1 freeze/thaw: 0.2 ng/mL  
Range: -3.5 to 4.6 ng/mL  
n: 15

Initial Specimen Conc. Measured on Day 0: 20.6 to 21.3 ng/mL  
Average Change in Conc. after 7 days at 2-8 degrees C.: -0.8 ng/mL  
Range: -1.1 to -0.7 ng/mL  
n: 3

Average Change in Conc. after 1 freeze/thaw: 0.9 ng/mL  
Range: -1.4 to 3.6 ng/mL  
n: 9

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

3. If testing is delayed more than 7 days, store frozen at less than or equal to -10 degrees C. (\(\leq -10\) degrees C.).

4. Specimens that are stored frozen must be mixed thoroughly after thawing to ensure consistency of results. Avoid repeated freezing and thawing.

5. Discard any remaining pretreated samples after testing is complete. ARCHITECT Sirolimus 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. 1L76 ARCHITECT Sirolimus Reagent Kit
2. 1L76-55 ARCHITECT Sirolimus 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. 1L76-01 ARCHITECT Sirolimus Calibrators
6. Vortex Mixer
7. 9527-26 XSYSTEMS CENTRIFUGE
8. Dry Block Heater and 2 Heater Blocks
9. ARCHITECT i PRE-TRIGGER SOLUTION
10. ARCHITECT i TRIGGER SOLUTION
11. ARCHITECT i WASH BUFFER
12. ARCHITECT i REACTION VESSELS
13. ARCHITECT i SEPTUM
14. ARCHITECT i REPLACEMENT CAPS
15. Pipettes and pipette tips

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

REAGENTS

REAGENT KIT, 100 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 SIROLIMUS REAGENT KIT (1L76)

1. MICROPARTICLES: 1 Bottle (8.0 mL) Anti-sirolimus (mouse, monoclonal) coated microparticles in MES buffer with protein (bovine) stabilizer. Minimum Concentration: 0.09% solids. Preservatives: sodium azide and ProClin 950.

2. CONJUGATE: 1 Bottle (8.0 mL) Sirolimus acridinium-labeled conjugate in citrate buffer. Minimum Concentration: 3.9 ng/mL. Preservative: ProClin 300.

3. ASSAY DILUENT: 1 Bottle (10.0 mL) Assay Diluent containing saline. Preservative: 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 Sirolimus 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, the reagents are stable until the expiration date.

3. The ARCHITECT Sirolimus 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. Recalibration 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.

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 are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

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 Sirolimus 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.

6. 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 Sirolimus calibration, test calibrators A, B, C, D, E, and F in replicates of two. Only one pretreated sample of each ARCHITECT Sirolimus 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 Sirolimus control must be tested to evaluate the assay calibration. Ensure that assay control values are within established ranges. Calibrators should be priority loaded.

   2. Calibration Range: 0.0 - 30.0 ng/mL. Once an ARCHITECT Sirolimus 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.

2. 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 Sirolimus 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 Sirolimus 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. To minimize the effects of evaporation all samples (specimens, calibrators and controls) must be tested within 3 hours of being placed on board the ARCHITECT i System.
   b. With the Transplant Pretreatment Tube, use the sample gauge to ensure sufficient patient specimen is present for the ARCHITECT Sirolimus 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. Values greater than 30.0 mcg/L are reported out as >30.0

MANUAL PRETREATMENT

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

Use only ARCHITECT Sirolimus Whole Blood Precipitation Reagent (1L76-55).

WARNING:

Only Transplant Pretreatment Tubes (LN 1P06-01) are acceptable when pretreating sirolimus 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 Sirolimus assay.

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 150 uL of each sample into a labeled XSYSTEMS Centrifuge Tube. Use a different centrifuge tube for each sample.

NOTE:

A new pipette tip must be used each time 150 uL is aspirated. Do not wipe pipette tip. Do not over-aspirate. Do not reuse pipette tips between replicates

3a. Set a precision pipette (repeat pipettor) to 300 uL. Aspirate enough of the ARCHITECT Sirolimus Whole Blood Precipitation Reagent from the yellow-labeled bottle. **NOTE: Do not process/dispense more than 4 tubes at a time.**

3b. Add 300 uL of ARCHITECT Sirolimus 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 immediately after capping each tube. (Use the maximum vortex setting).

WARNING:

Failure to vortex each tube immediately after addition of the ARCHITECT Sirolimus 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 Sirolimus Whole Blood Precipitation Reagent into all the tubes at once. Each individual tube must be capped and vortexed immediately after addition of the ARCHITECT Sirolimus Whole Blood Precipitation Reagent before adding ARCHITECT Sirolimus Whole Blood Precipitation Reagent to the subsequent tubes.

4. Load each tube into a heating block set at 42 degrees C. Incubate for 10 minutes and centrifuge immediately.

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

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

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

NOTE:

Use a different Transplant Pretreatment Tube for each sample.

WARNING:

Only Transplant Pretreatment Tubes (LN 1P06-01) are acceptable when pretreating sirolimus 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 Sirolimus assay.

WARNING:

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

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

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

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

NOTE:

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

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

NOTE: The first batch of extractions for the day must include both control levels. For each subsequent extraction batch, one level of control is to be included in each batch.

X. RESULTING/REPORTABLE RANGE

RESULTS

The ARCHITECT Sirolimus assay uses a 4 Parameter Logistic Curve Fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.
ANALYTICAL MEASUREMENT RANGE (REPORTABLE RANGE)

The measurement range for the ARCHITECT Sirolimus assay is 1.0 µg/L (minimum reportable value based on Analytical Sensitivity) to 30 µg/L.

Values below 1.0 µg/L are reported as <1.0.
Values above 30.0 are reported as >30.0.

XI. EXPECTED VALUES

6.0 to 15.0 µg/L.

Optimal sirolimus concentration 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 cross-reactivity with metabolites, nor should correction factors be applied. Laboratories should include identification of the assay used in order to aid in interpretation of results.

Optimal ranges depend upon the patient's clinical state, individual differences in sensitivity to immunosuppressive and adverse effects of sirolimus, coadministration of other immunosuppressants, time post-transplant, and a number of other factors. Therefore, individual sirolimus 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 institution should establish the optimal ranges based on the specific assay used and other factors relevant to their patient population prior to reporting patient results.

Drug Trials 301 and 302 initially established the safety and efficacy of sirolimus immunosuppressive therapy in conjunction with full-dose cyclosporine and corticosteroids. These randomized, double blind trials were conducted with 1295 post renal transplant enrollees. Patients who received sirolimus were given daily doses of 2 mg or 5 mg following an initial loading dose that was three times the maintenance dose. Mean sirolimus whole blood trough concentrations through month 6 following transplantation, as measured by the IMx Sirolimus assay, were 9 ng/mL (range 4.5 - 14 ng/mL [10th to 90th percentile]) for the 2 mg/day treatment group, and 17 ng/mL (range 10 - 28 ng/mL [10th to 90th percentile]) for the 5 mg/day treatment group.

A study was conducted to assess the safety and efficacy of sirolimus as a maintenance regimen following cyclosporine withdrawal at 3 to 4 months post renal transplantation. This randomized, multicenter study compared patients who were administered sirolimus, cyclosporine, and corticosteroids continuously with patients who received the same standardized therapy for the first 3 months after transplantation (prerandomization period) followed by the withdrawal of cyclosporine. During cyclosporine withdrawal, the sirolimus dosages were adjusted to achieve targeted sirolimus whole blood trough concentration ranges of 20 to 30 ng/mL through month 12, and 15 to 25 ng/mL thereafter (as measured by the IMx Sirolimus assay). At 3 months, 430 patients were equally randomized to either sirolimus with cyclosporine therapy or sirolimus as a maintenance regimen following cyclosporine withdrawal. Further analysis of the IMx Sirolimus data in this study found that during months 4 through 12 following transplantation, the mean sirolimus whole blood trough concentrations were 10.7 ng/mL (range 6.5 - 15.0 ng/mL [10th to 90th percentile]) in the sirolimus and cyclosporine treatment group (n = 215), and were 23.3 ng/mL (range 16.9 - 29.3 ng/mL [10th to 90th percentile]) in the cyclosporine withdrawal treatment group (n = 215).

XII. 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 sirolimus results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. The concentration of sirolimus 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. This cross-reactivity can lead to a positive bias in patient results when compared with methods that are specific for the parent molecule (e.g. Liquid Chromatography Mass Spectrometry/Mass Spectrometry [LC/MS/MS]). Refer to the SPECIFICITY section below for estimates of cross-reactivity of ARCHITECT Sirolimus to some metabolites of sirolimus. Refer to the METHOD COMPARISON section below for representative data comparing patient results from the ARCHITECT Sirolimus assay to the IMx Sirolimus 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 Sirolimus) that employ mouse monoclonal antibodies.

XIII. SPECIFICITY/INTERFERENCES

INTERFERENCE

The ARCHITECT Sirolimus assay is designed to have a mean recovery of 100 +/- 10% in the presence of the pharmaceutical substances and potential interfering substances listed below.

Potential interference was evaluated by a study based on guidance from the CLSI document EP7-A2. Whole blood specimens were supplemented with various drugs and potentially interfering compounds (triglycerides, hematocrit, bilirubin, total protein, cholesterol, uric acid, HAMA and rheumatoid factor [RF]) at levels indicated in the following tables. The average recovery observed during the study ranged from 95 to 106%.

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<tr>
<td>Acetaminophen</td>
<td>200 µg/mL</td>
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<td>Amikacin*2H2O</td>
<td>150 µg/mL</td>
<td>Lidocaine</td>
<td>60 µg/mL</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>5.8 µg/mL</td>
<td>Lovastatin</td>
<td>20 µg/mL</td>
</tr>
<tr>
<td>Apresoline</td>
<td>9.6 µg/mL</td>
<td>Minoxidil</td>
<td>60 µg/mL</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>10 µg/mL</td>
<td>Micophenolic Acid</td>
<td>25 µg/mL</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>1.5 µg/mL</td>
<td>Micophenolic Acid</td>
<td>1800 µg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucuronide</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>120 µg/mL</td>
<td>N-Acetyl-Procainamide</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>840 µg/mL</td>
<td>Nadolol</td>
<td>1.2 µg/mL</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>250 µg/mL</td>
<td>Nicardipine</td>
<td>0.5 µg/mL</td>
</tr>
<tr>
<td>Chorquirine</td>
<td>1.5 µg/mL</td>
<td>Penicillin G Na+</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100 µg/mL</td>
<td>Phenobarbital</td>
<td>150 µg/mL</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>7.4 µg/mL</td>
<td>Phenyoit</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.01 µg/mL</td>
<td>Prazosin</td>
<td>25 µg/mL</td>
</tr>
<tr>
<td>Colchicicine</td>
<td>0.09 µg/mL</td>
<td>Prednisolone</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Cortisone</td>
<td>1.2 µg/mL</td>
<td>Prednisone</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>3.2 µg/mL</td>
<td>Primidone</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Dibutin</td>
<td>0.08 µg/mL</td>
<td>Probucol</td>
<td>600 µg/mL</td>
</tr>
<tr>
<td>Digoxin</td>
<td>0.0048 µg/mL</td>
<td>Procainamide</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>0.04 µg/mL</td>
<td>Propranolol</td>
<td>5 µg/mL</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>30 µg/mL</td>
<td>Quindine</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>200 µg/mL</td>
<td>Ranitidine</td>
<td>200 µg/mL</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>75 µg/mL</td>
<td>Rifampin</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>240 µg/mL</td>
<td>Spectinomycin</td>
<td>100 ng/mL</td>
</tr>
<tr>
<td>Substance</td>
<td>Concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------</td>
<td>---------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluorocytosine</td>
<td>40 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.06 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>20 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>150 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ganciclovir</td>
<td>100 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>20 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>100 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>40 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>120 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valproic Acid</td>
<td>144.2 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>1.2 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>60 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>50 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verapamil</td>
<td>10 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanamycin A Sulfate</td>
<td>60 µg/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Potential Interfering Substance

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>1500 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>
<tr>
<td>HAMA</td>
<td>14.5 – 340 ng/mL</td>
</tr>
<tr>
<td>RF</td>
<td>20.9 – 445 IU/mL</td>
</tr>
</tbody>
</table>

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

### XIV. SPECIFIC PERFORMANCE CHARACTERISTICS

#### Precision

The ARCHITECT Sirolimus assay is designed to have a precision of ≤ 10% total CV. A study was performed with the ARCHITECT Sirolimus assay based on guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) document EP5-A2.18 Abbott Immunosuppressant-MCC (levels 1 and 2) 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</td>
<td>80</td>
<td>3.8</td>
<td>0.1</td>
<td>3.0</td>
<td>0.3</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>3.6</td>
<td>0.1</td>
<td>3.6</td>
<td>0.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Level 2</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>13.2</td>
<td>0.3</td>
<td>2.6</td>
<td>0.6</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>12.1</td>
<td>0.3</td>
<td>2.4</td>
<td>0.7</td>
<td>5.5</td>
</tr>
<tr>
<td>Panel 1</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>8.1</td>
<td>0.2</td>
<td>2.6</td>
<td>0.4</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>7.8</td>
<td>0.2</td>
<td>3.0</td>
<td>0.4</td>
<td>5.2</td>
</tr>
<tr>
<td>Panel 2</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>15.3</td>
<td>0.3</td>
<td>2.3</td>
<td>0.7</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>14.7</td>
<td>0.4</td>
<td>3.0</td>
<td>0.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Panel 3</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>5.0</td>
<td>0.2</td>
<td>3.1</td>
<td>0.2</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>4.8</td>
<td>0.2</td>
<td>3.5</td>
<td>0.3</td>
<td>5.7</td>
</tr>
<tr>
<td>Panel 4</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>11.2</td>
<td>0.4</td>
<td>4.0</td>
<td>0.5</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>10.7</td>
<td>0.3</td>
<td>2.6</td>
<td>0.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Panel 5</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>21.9</td>
<td>0.5</td>
<td>2.3</td>
<td>0.8</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>20.9</td>
<td>0.7</td>
<td>3.2</td>
<td>0.1</td>
<td>5.2</td>
</tr>
</tbody>
</table>

#### Specificity

A study was performed with the ARCHITECT Sirolimus assay based on guidance from the CLSI document EP7-A2.19 Aliquots of whole blood specimens were augmented with sirolimus, targeting values ranging from 5 to 22 ng/mL. These five specimens were spiked with cross-reactant solution. Data from this study are summarized in the following table.* HPLC fractions with identifiable species of sirolimus metabolites that have been detected in human whole blood specimens were tested in.
the ARCHITECT Sirolimus assay. Purified sirolimus metabolites are not commercially available for cross-reactivity testing. Sirolimus metabolites were prepared in vitro by incubating sirolimus with CYP450-3A4. The crude mixture was purified by normal phase chromatography on a silica gel flash column, followed by a second fractionation by reverse phase HPLC.

<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&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2 (41-0-demethyl-hydroxyl-sirolimus)</td>
<td>10</td>
<td>0.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.7</td>
</tr>
<tr>
<td>F3 (41-0-demethyl-hydroxyl-sirolimus; 7-0-demethyl-sirolimus)</td>
<td>3</td>
<td>0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.6</td>
</tr>
<tr>
<td>F4 (11-hydroxy-sirolimus)</td>
<td>10</td>
<td>3.7</td>
<td>36.8</td>
</tr>
<tr>
<td>F5 (41-0-demethyl-sirolimus)</td>
<td>10</td>
<td>2.0</td>
<td>20.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cross-reactivities as estimated by interference with the measurement of sirolimus in whole blood specimens.

<sup>b</sup> Concentration is below the reportable range of the ARCHITECT Sirolimus assay.

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

**Sensitivity**

The limit of detection of the ARCHITECT Sirolimus assay, defined as the concentration at two standard deviations above the ARCHITECT Sirolimus Calibrator A (0.0 ng/mL) was calculated to be 1.0 ng/mL<sup>*</sup> 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.

**XV. ALTERNATE METHODS**

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

**XVI. REFERENCES**

ABBOTT ARCHITECT Sirolimus package insert
Abbott Laboratories
Diagnostics Division
Abbott Park, IL 60064
PN 290-512 6/07
Revision 09/07