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

- **REF**: List Number
- **IVD**: Lot Number
- **Store at 2-8°C**: Expiration Date
- **Store at 15-30°C**: Control Number
- **Serial Number**: Assay CD-ROM
- **Consult Instructions for use**: Reaction Vessels
- **Serial Number**: Septum
- **Serial Number**: Replacement Caps
- **Serial Number**: Centrifuge Tubes
- **Authorized Representative**: Sterile, method of sterilization using irradiation
- **Manufacturer**: Do not re-use

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.
Sirolimus is a mammalian target of rapamycin (mTOR) inhibitor. It is a macrocyclic lactone that binds to the immunosuppressive protein 12, and the resulting complex binds to a specific cell-cycle regulatory protein in mammalian target of rapamycin, 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 inhibits the immunophilin FK-binding protein 12, and the resulting complex binds to specific cell-cycle regulatory proteins. 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 immunosuppressive activity of sirolimus metabolites is thought to be due to the oral solution. Ascending dose studies (range 0.5 - 6.5 mg/m$^2$/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 between methods that are specific for the parent drug and the metabolites is consistent between patients. For a small number of patients using HPLC/MS/MS suggests that the steady-state profile of sirolimus and the metabolites is consistent between 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.

Among 30 stable renal allograft recipients who received a 14-day course of sirolimus concurrently with cyclosporine and corticosteroids, there was a 4.5-fold difference in apparent mean drug clearance of 208 ± 99 mL/hr/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-desmethylation 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 due to the oral solution. Ascending dose studies (range 0.5 - 6.5 mg/m$^2$/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 between methods that are specific for the parent drug and the metabolites is consistent between patients. For a small number of patients using HPLC/MS/MS suggests that the steady-state profile of sirolimus and the metabolites is consistent between 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.

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

• 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 beginning use of 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.

• Sephams 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 invert 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.

Storage Instructions
• The ARCHITECT Sirolimus 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 Sirolimus Reagent Kit may be stored on the ARCHITECT System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. Recalibration may be required to obtain maximum on-board 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 septum covers) 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. For information on unloading reagents/kit to the ARCHITECT System Operations Manual, Section 6.

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 Sirolimus assay file must be installed on the ARCHITECT System from the ARCHITECT System CD-ROM Addition A before performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

• 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.

• The default result unit for the ARCHITECT Sirolimus assay is ng/mL. When the alternate result unit, μg/L, is selected, the conversion factor used by the system is 1.0939. When the alternate result unit, μg/L, is selected, the conversion factor used by the system is 1.0.
• Conversion Formula: (Concentration in ng/mL) x 1.0939 = μg/L
• Conversion Formula: (Concentration in ng/mL) x 1.0 = μg/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

**Specimen Types**
• Only human whole blood specimens collected in EDTA tubes may be used with the ARCHITECT Sirolimus assay. Follow the manufacturer’s instructions for whole blood collection tubes.

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

• The ARCHITECT 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.

**Specimen Conditions**
• Do not use specimens with the following conditions:
  - heat-inactivated specimens
  - cadaver specimens or any other body fluids
  - obvious microbial contamination

Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis
• Follow the tube manufacturer’s processing instructions for whole blood collection tubes.

• Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.

• Follow the Manual Pretreatment Procedure in the PROCEDURE section.

Storage
• ARCHITECT Sirolimus values may shift during 2-8°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°C or after 1 freeze/thaw cycle shown as a concentration change from Day 0.*

<table>
<thead>
<tr>
<th>Initial Specimen Conc. Measured (Range)</th>
<th>Average Change on Day 0</th>
<th>Average Change on Day 0 (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 ng/mL</td>
<td>-0.9 to 1.1 ng/mL</td>
<td>-1.1 to 1.5 ng/mL</td>
</tr>
<tr>
<td>0.9 ng/mL</td>
<td>-0.8 to 1.1 ng/mL</td>
<td>-1.1 to 1.5 ng/mL</td>
</tr>
<tr>
<td>2.7 ng/mL</td>
<td>-0.4 to 1.1 ng/mL</td>
<td>-1.1 to 1.5 ng/mL</td>
</tr>
<tr>
<td>5.3 to 7.3 ng/mL</td>
<td>-1.1 to 1.5 ng/mL</td>
<td>-1.1 to 1.5 ng/mL</td>
</tr>
<tr>
<td>12.1 to 15.9 ng/mL</td>
<td>-0.8 to 1.5 ng/mL</td>
<td>-0.8 to 1.5 ng/mL</td>
</tr>
<tr>
<td>20.6 to 23.1 ng/mL</td>
<td>-0.8 to 1.5 ng/mL</td>
<td>-0.8 to 1.5 ng/mL</td>
</tr>
</tbody>
</table>

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

• 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.

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

• If testing is delayed more than 7 days, store frozen at less than or equal to -10°C (-10°C).

• Specimens or reagents should not be mixed thoroughly after thawing to ensure consistency of results. Avoid repeated freezing and thawing.

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3
Manual Pretreatment Procedure

The ARCHITECT Sirolimus assay requires a manual pretreatment step for all whole blood specimen samples. Use only ARCHITECT Sirolimus Whole Blood Precipitation Reagent (1L76-55).

1. Mix each sample (specimen, calibrator, or control) thoroughly by slow inversion of the container 5-10 times. Older whole blood specimens may take a longer mixing time. Visual inspection is recommended to assure the specimen is adequately mixed.

2. Precision pipettes 150 μL of each sample into an XSYSTEMS Centrifuge Tube. Use a different tube for each sample.

Note: A new pipette tip must be used each time 150 μL is aspirated. Do not wipe pipette tip. Do not use aspirate. Do not reuse pipette tips between replicates. The practice of pre-wetting the tip and reverse pipetting are not recommended, since they may generate error codes and add greater imprecision to the assay.

3a. Set a precision pipette to 330 μL. Aspirate with a precision pipette 330 μL of the ARCHITECT Sirolimus Whole Blood Precipitation Reagent from the yellow-labeled bottle.

3b. Add 300 μL of ARCHITECT Sirolimus 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 Precipitation Reagent before adding Precipitation Reagent to subsequent tubes.

3c. Cap the tube and vortex immediately.

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 homogenous. 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 displace 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 to the contents of the next centrifuge tube.

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

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

Manual Pretreatment Procedure

Attention: To obtain optimal results for the ARCHITECT Sirolimus assay, the Manual Pretreatment Steps listed below must be followed precisely.
• Before loading the ARCHITECT Sirolimus Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend the 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 Sirolimus Reagent Kit on the ARCHITECT i System.
• Verify that all necessary assay reagents are present.
• Ensure that septums are present on all reagent bottles.
• Order calibration, if necessary.
• For information on ordering calibrators, refer to the ARCHITECT System Operations Manual, Section 5.
• Order tests.
• For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
• No more than 3 replicates may be sampled from the same Transplant Calibrator.
• 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.
• With the Transplant Pretreatment Tube, use the sample gauge to ensure sufficient patient specimen is present for the ARCHITECT Sirolimus assay.
• Verify that all necessary assay reagents are present.
• Refer to 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 Sirolimus calibration, test calibrators A, B, C, D, E, and F in replicates of two. Only one pretested 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.
• 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 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 Sirolimus 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 QC kit 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 should not be used and must be rejected. Recalibration may be indicated.

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT Sirolimus assay belongs to method group 6. ARCHITECT Sirolimus Calibrators may be used when MasterCheck is not available. Refer to the ARCHITECT System Operations Manual, Appendix B.

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

Flags
Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 9.

Measurement Range (Reportable Range)
The measurement range for the ARCHITECT Sirolimus assay is 2 ng/mL (minimum reportable value based on Functional Sensitivity) to 30 ng/mL.

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 sirolimus results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
• 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.

Specimen Dilution Procedures
Specimens with a sirolimus 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 Sirolimus assay is 1:2.
  • 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.
  • Add 150 μL of the patient specimen to 150 μL of ARCHITECT Sirolimus Calibrator A, then proceed with the Manual Pretreatment Procedure in the PROCEDURE section.

• For diagnostic purposes, results should be used in conjunction with other data, e.g., symptoms, results of other tests, clinical impressions, etc.

• If the sirolimus results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

• 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.
• Immunosuppressants are non-specific 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.

• Heterophilic antibodies in human serum can react with reagents containing human IgG or IgM. 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.

• 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.

EXPECTED VALUES

CAUTION: 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 were administered 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. 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 8.5 - 15.0 ng/mL [10th to 90th percentile]) in the sirolimus and cyclosporine treatment group (n=215), and were 23.3 ng/mL (range 16.8 - 36.3 ng/mL [10th to 90th percentile]) in the cyclosporine withdrawal treatment group (n=215).

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Sirolimus assay is designed to have a precision of ±15% (±1 CV).

A study was performed with the ARCHITECT Sirolimus assay based on guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) document EP2-A2.** 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>Specimen</th>
<th>Concentration (ng/mL)</th>
<th>Recovery</th>
<th>Concentration (ng/mL)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0</td>
<td>3.0</td>
<td>2.7</td>
<td>90</td>
</tr>
<tr>
<td>B</td>
<td>0.0</td>
<td>3.0</td>
<td>2.9</td>
<td>97</td>
</tr>
<tr>
<td>C</td>
<td>0.0</td>
<td>3.0</td>
<td>2.9</td>
<td>97</td>
</tr>
<tr>
<td>D</td>
<td>0.0</td>
<td>3.0</td>
<td>2.7</td>
<td>90</td>
</tr>
<tr>
<td>E</td>
<td>0.0</td>
<td>3.0</td>
<td>2.5</td>
<td>83</td>
</tr>
</tbody>
</table>

** Mean Recovery: 91%

A 0.0 21.0 19.2 91
B 0.0 21.0 19.0 90
C 0.0 21.0 19.9 95
D 0.0 21.0 19.5 93
E 0.0 21.0 19.9 95

** Mean Recovery: 95%

A 0.0 27.0 23.2 66
B 0.0 27.0 25.8 96
C 0.0 27.0 24.4 90
D 0.0 27.0 26.1 97
E 0.0 27.0 27.8 103

** Mean Recovery: 94%

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

Recovery

The ARCHITECT Sirolimus assay is designed to have a mean recovery of 100 ± 10% of the expected value.

A study was performed where known concentrations of sirolimus were added to 15 aliquots of whole blood specimens. The concentration of sirolimus was determined using the ARCHITECT Sirolimus assay and the resulting percent recovery was calculated. Data from this study are summarized in the following table.*

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Concentration (ng/mL)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0</td>
<td>3.0</td>
</tr>
<tr>
<td>B</td>
<td>0.0</td>
<td>3.0</td>
</tr>
<tr>
<td>C</td>
<td>0.0</td>
<td>3.0</td>
</tr>
<tr>
<td>D</td>
<td>0.0</td>
<td>3.0</td>
</tr>
<tr>
<td>E</td>
<td>0.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

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A 0.0 21.0 19.2 91
B 0.0 21.0 19.0 90
C 0.0 21.0 19.9 95
D 0.0 21.0 19.5 93
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E 0.0 27.0 27.8 103

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

ARCHITECT Sirolimus assay to the IMx Sirolimus assay and an LC/MS/MS method.

Each institution should establish the optimal ranges based on 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.

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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 maintenance regimen following cyclosporine withdrawal at 3 to 4 months post renal transplantation. This randomized, multicenter study compared 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 8.5 - 15.0 ng/mL [10th to 90th percentile]) in the sirolimus and cyclosporine treatment group (n=215), and were 23.3 ng/mL (range 16.8 - 36.3 ng/mL [10th to 90th percentile]) in the cyclosporine withdrawal treatment group (n=215).
Dilution Linearity

The ARCHITECT Sirolimus 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 sirolimus whole blood specimens with the ARCHITECT Sirolimus Calibrator A. The concentration of sirolimus was determined for each dilution of sample and the mean percent (%) recovery was calculated. Data from this study is summarized in the following table.*

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Dilution Factor</th>
<th>Diluted Concentration (ng/mL)</th>
<th>Calculated Concentration* (ng/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:1.11</td>
<td>20.1</td>
<td>20.3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:1.25</td>
<td>20.2</td>
<td>20.4</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>1:1.43</td>
<td>20.3</td>
<td>20.4</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>1:2.50</td>
<td>20.8</td>
<td>21.1</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>1:5.00</td>
<td>21.5</td>
<td>21.9</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>1:10.00</td>
<td>25.7</td>
<td>26.1</td>
<td>115</td>
</tr>
</tbody>
</table>

* Calculated Concentration = Observed Concentration x Dilution Factor

% Recovery = (Observed Concentration x Dilution Factor) / Undiluted Observed Concentration

Specificity

A study was performed with the ARCHITECT Sirolimus assay based on guidance from the CLSI document EP7-A2.* All lots of whole blood specimens were supplemented with sirolimus, targeting values ranging from 5 to 22 ng/mL. These five specimens were spiked with cross-reactant solution. Data from this study is summarized in the following table.*

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Test Conc. (μg/mL)</th>
<th>Mean Excess Concentration (ng/mL)</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirolimus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 (41'-0-demethyl-sirolimus)</td>
<td>10</td>
<td>0.87*</td>
<td>8.7</td>
</tr>
<tr>
<td>F2 (41'-0-demethyl-hydroxysiroli-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m)</td>
<td>10</td>
<td>0.12*</td>
<td>7.6</td>
</tr>
<tr>
<td>F3 (41'-0-demethyl-hydroxyl-siroli-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m)</td>
<td>10</td>
<td>3.7</td>
<td>36.8</td>
</tr>
<tr>
<td>F4 (11-hydroxy-sirolimus)</td>
<td>10</td>
<td>10.0</td>
<td>20.3</td>
</tr>
</tbody>
</table>

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

Functional Sensitivity

The ARCHITECT Sirolimus assay is designed to have a functional sensitivity of ≤ 2 ng/mL, which is below the reportable range of the ARCHITECT Sirolimus assay. In a study, whole blood specimens spiked with cross-reactant substances listed below 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>Interference</th>
<th>Test Compound Test Conc. (μg/mL)</th>
<th>Mean Excess Concentration (ng/mL)</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirolimus</td>
<td>10</td>
<td>0.87*</td>
<td>8.7</td>
</tr>
<tr>
<td>Cross-reactant substances</td>
<td>10</td>
<td>0.12*</td>
<td>7.6</td>
</tr>
<tr>
<td>F3 (41'-0-demethyl-sirolimus-m)</td>
<td>10</td>
<td>3.7</td>
<td>36.8</td>
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<td>F4 (11-hydroxy-sirolimus)</td>
<td>10</td>
<td>10.0</td>
<td>20.3</td>
</tr>
</tbody>
</table>

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

Sensitivity

The ARCHITECT Sirolimus assay is designed to have a limit of detection of ≤ 1 ng/mL, which is below the reportable range of the ARCHITECT Sirolimus assay. The limit of detection of the ARCHITECT Sirolimus assay, defined as the concentration at which 95% of the dilution linearity study values fall within ±10% of the expected results for diluted samples, was determined for each dilution of specimen. 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 Sirolimus assay value exhibiting a 20% CV was calculated to be 0.7 ng/mL.*

1 Undiluted 27.8
1:1.11 24.3 27.0 97
1:1.25 21.2 26.5 95
1:1.43 20.4 29.2 105
1:1.67 17.7 26.8 106
1:2.50 11.9 29.8 107
1:5.00 6.6 30.0 119
1:10.00 3.3 33.0 118

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

Recovery

The ARCHITECT Sirolimus assay is designed to have a mean recovery of 95 to 106%.* A study was performed with the ARCHITECT Sirolimus assay based on guidance from the CLSI document EP7-A2.* All lots of whole blood specimens were supplemented with sirolimus, targeting values ranging from 5 to 22 ng/mL. These five specimens were spiked with cross-reactant solution. Data from this study is summarized in the following table.*

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Dilution Factor</th>
<th>Undiluted 27.8</th>
<th>Diluted 25.3</th>
<th>Calculated Concentration* (ng/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:1.11</td>
<td>20.1</td>
<td>20.6</td>
<td>20.2</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>1:1.25</td>
<td>20.2</td>
<td>23.3</td>
<td>23.1</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>1:1.43</td>
<td>20.3</td>
<td>25.6</td>
<td>25.5</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>1:2.50</td>
<td>24.2</td>
<td>24.8</td>
<td>24.7</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1:5.00</td>
<td>25.5</td>
<td>25.5</td>
<td>25.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:10.00</td>
<td>27.0</td>
<td>27.0</td>
<td>27.0</td>
<td>109</td>
</tr>
</tbody>
</table>

* Calculated Concentration = Observed Concentration x Dilution Factor

% Recovery = (Observed Concentration x Dilution Factor) / Undiluted Observed Concentration

Reactivity

The ARCHITECT Sirolimus assay is designed to have a mean recovery of 95 to 106%.* A study was performed with the ARCHITECT Sirolimus assay based on guidance from the CLSI document EP7-A2.* All lots of whole blood specimens were supplemented with sirolimus, targeting values ranging from 5 to 22 ng/mL. These five specimens were spiked with cross-reactant solution. Data from this study is summarized in the following table.*

<table>
<thead>
<tr>
<th>Interference</th>
<th>Metabolite</th>
<th>Added Concentration (ng/mL)</th>
<th>Detected Concentration (ng/mL)</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirolimus</td>
<td>F1</td>
<td>10</td>
<td>0.87*</td>
<td>8.7</td>
</tr>
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<td>Cross-reactant substances</td>
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<td>7.6</td>
<td></td>
</tr>
<tr>
<td>F3 (41'-0-demethyl-sirolimus-m)</td>
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<td>3.7</td>
<td>36.8</td>
<td></td>
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<td>F4 (11-hydroxy-sirolimus)</td>
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<td>20.3</td>
<td></td>
</tr>
</tbody>
</table>

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

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%.*

<table>
<thead>
<tr>
<th>Interference</th>
<th>Test Compound Test Conc. (μg/mL)</th>
<th>Mean Excess Concentration (ng/mL)</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
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<td>10.0</td>
<td>20.3</td>
</tr>
</tbody>
</table>

* Cross-reactivities as estimated by interference with the measurement of sirolimus in whole blood specimens.
**Method Comparison**

The ARCHITECT Sirolimus assay is designed to have a correlation coefficient of ≥ 0.90 for specimens between 2 - 30 ng/mL when compared to IMx Sirolimus. A study was performed using human whole blood specimens stored at -10°C or colder from renal transplant patients receiving sirolimus therapy, where regression analysis was performed using the Passing-Bablok method. Data from this study are summarized in the following table.*

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Test Conc. (ng/mL)</th>
<th>Test Compound</th>
<th>Test Conc. (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydropyridine</td>
<td>200 μg/mL</td>
<td>Ramipril</td>
<td>200 μg/mL</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>75 μg/mL</td>
<td>T sinapine</td>
<td>50 μg/mL</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>240 μg/mL</td>
<td>Spectramycin</td>
<td>100 μg/mL</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>40 μg/mL</td>
<td>Tacrolimus</td>
<td>0.05 μg/mL</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>20 μg/mL</td>
<td>Tiotropium</td>
<td>150 μg/mL</td>
</tr>
<tr>
<td>Ganciclovir</td>
<td>100 μg/mL</td>
<td>Tobramycin</td>
<td>20 μg/mL</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>100 μg/mL</td>
<td>Trimethoprim</td>
<td>40 μg/mL</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>120 μg/mL</td>
<td>Valproic Acid</td>
<td>144 μg/mL</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>1.2 μg/mL</td>
<td>Vancomycin</td>
<td>60 μg/mL</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>50 μg/mL</td>
<td>Venepamil</td>
<td>10 μg/mL</td>
</tr>
<tr>
<td>Karbamyl</td>
<td>60 μg/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Potential Interfering Substance**

- Tripterygium
- Hemacetin ± 25%, ± 55%
- Bilirubin | 40 mg/dL |
- Total Protein | 2 g/dL |
- Total Protein | 12 g/dL |
- Cholesterol | 500 mg/dL |
- Uric Acid | 20 mg/dL |
- HAMA | 14.5 – 340 ng/mL |
- RF | 20.9 – 445 IU/mL |

**Additional testing of the above samples was completed with LC/MS/MS.**

A bias analysis of the ARCHITECT Sirolimus vs. IMx Sirolimus assay was performed on the same 167 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 Sirolimus vs. IMx Sirolimus assay in this study was 0.46 ng/mL. The 95% confidence interval of the ng/mL difference bias is 0.20 ng/mL to 0.72 ng/mL. Results of the study are summarized below.

**ARCHITECT Sirolimus ng/mL Difference**

<table>
<thead>
<tr>
<th>Bias from IMx Sirolimus</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG/mL Difference</td>
</tr>
</tbody>
</table>

**ARCHITECT Sirolimus ng/mL Difference**

<table>
<thead>
<tr>
<th>Bias from LC/MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG/mL Difference</td>
</tr>
</tbody>
</table>

**BIBLIOGRAPHY**


