Revised September 2015.

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

NAME

ARCHITECT Sirolimus

INTENDED USE

The ARCHITECT Sirolimus assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of sirolimus in human whole blood on the ARCHITECT iSystem.

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 THE 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)[1] 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 In this study, clinical outcomes of patients withdrawn from cyclosporine and maintained on sirolimus and corticosteroids compared favorably to patients continuing on the triple-drug immunosuppressive regimen.[2] Because of potential toxic effects associated with high trough levels of sirolimus, therapeutic drug monitoring of sirolimus immunosuppressive therapy has been recommended.[1] Sirolimus is a macrocyclic lactone fermentation product of *Streptomyces hygroscopicus*, first discovered in the soil of Rapa Nui (Easter Island).[3] 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 G_1 to the S phase of the cell cycle.[4] Pharmacokinetic studies indicate that sirolimus is primarily sequestered in erythrocytes, and that the appropriate sample medium with which to monitor sirolimus is whole blood.[5]

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.[6] 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.[7] A good correlation (r^2 = 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.[7]

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.[7] 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.[1] 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.[4] 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.[5] 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.[8] Consistency in metabolite profiles should contribute to a good correlation between methods that are specific for the parent drug and the methods that detect both parent drug and its metabolites.[9, 10]

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 iSystem.

1. Sample, assay diluent, and anti-sirolimus coated paramagnetic microparticles are combined to create a reaction mixture. The sirolimus present in the sample binds to the anti-sirolimus coated microparticles.
2. 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.
3. Following an incubation, the microparticles are washed, and pre-trigger and trigger solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an indirect relationship between the amount of sirolimus in the sample and the RLUs detected by the ARCHITECT iSystem optics.

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

REAGENTS

Kit Contents

ARCHITECT Sirolimus 1L76

REF	1L76-25
Σ	100
MICROPARTICLES	1 x 8.0 mL
CONJUGATE	1 x 8.0 mL
ASSAY DILUENT	1 x 10.0 mL

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

CONJUGATE Sirolimus acridinium-labeled conjugate in citrate buffer. Minimum concentration: 3.9 ng/mL. Preservatives: ProClin 300.

ASSAY DILUENT Assay Diluent containing saline. Preservative: ProClin 300.

Other Reagents

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

Warnings and Precautions

- IVD
- 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.[11-14]

The following warnings and precautions apply to: MICROPARTICLES / CONJUGATE / ASSAY DILUENT

WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

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

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may 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 an open 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 and 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.

Reagent Storage

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

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8 °C	Until expiration date	May be used immediately after removal from 2-8 °C storage. Store in upright position.
On board	System temperature	30 days	Discard after 30 days. 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.

* Reagents may be stored on or off the ARCHITECT iSystem. 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 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, 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 are 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 iSystem prior to performing the assay.

For detailed information on assay file installation and 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.

Alternate Result Units

Edit assay parameter "Result concentration units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default result unit	Conversion factor	Alternate result unit
ng/mL	1.0939	nmol/L
	1.0	µg/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Specimen Type	Collection Tube
Whole blood	EDTA

- The instrument does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.
- It is recommended that specimens be labeled with both the time of collection as well as the last drug administration.

Specimen Conditions

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

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Whole Blood	2-8 ˚C	≤ 7 days

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

Initial Specimen Concentration Measured on Day 0	Average Change in Concentration after 7 days at 2-8 ˚C	Average Change in Concentration after 1 freeze/thaw
5.3 to 7.3 ng/mL	0.1 ng/mL (Range -0.4 to 1.1 ng/mL) n = 4	0.4 ng/mL (Range -0.9 to 1.1 ng/mL) n = 12
12.1 to 15.9 ng/mL	-1.1 ng/mL (Range -4.4 to 0.8 ng/mL) n = 5	0.2 ng/mL (Range -3.5 to 4.6 ng/mL) n = 15
20.6 to 21.3 ng/mL	-0.8 ng/mL (Range -1.1 to -0.7 ng/mL) n = 3	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.

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

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

- 1L76 ARCHITECT Sirolimus Reagent Kit
- 1L76-55 ARCHITECT Sirolimus Whole Blood Precipitation Reagent
- 1P06 Transplant Pretreatment Tubes

Materials Required but not Provided

- ARCHITECT Sirolimus Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 1L76-01 ARCHITECT Sirolimus Calibrators
- Commercial Controls
- Vortex Mixer
- Laboratory microcentrifuge
- Polypropylene Centrifuge Tubes compatible with laboratory microcentrifuge.
- Dry Block Heater and 2 Heater Blocks
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

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

Manual Pretreatment Procedure

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

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

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 in this package insert.

Warning: Only Transplant Pretreatment Tubes (LN 1P06) are acceptable when pretreating sirolimus samples for use on the ARCHITECT iSystem. 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.

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 pipette 150** µL of each sample into a microcentrifuge tube or equivalent polypropylene centrifuge tube (e.g. round bottom) immediately after mixing. 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 over-aspirate. Do not reuse pipette tips between replicates. The use of positive displacement pipettes, 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 **300 µL. Aspirate** with a precision pipette, **300 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 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°C**. Incubate for **10** minutes and centrifuge immediately.

   Warning: Failure to incubate the sample will lead to erroneous assay results.
5. **Load** each tube into a microcentrifuge 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 a minimum of 4 minutes at > 9500 x g RCF, or 38,500 g-minutes.
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 **Transplant Pretreatment Tube**, when the ARCHITECT iSystem is ready to accept samples.

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

Warning: Only Transplant Pretreatment Tubes (LN 1P06) are acceptable when pretreating sirolimus samples for use on the ARCHITECT iSystem. 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.

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.

Assay Procedure

- Before loading the 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.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert 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 about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- 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 Pretreatment Tube.
  - 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 iSystem.
  - With the Transplant Pretreatment Tube, use the sample gauge to ensure sufficient patient specimen is present for the ARCHITECT Sirolimus assay.
- Prepare calibrators and controls.
  - Refer to the Manual Pretreatment 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 sirolimus value exceeding 30.0 ng/mL are flagged with the code "> 30.0 ng/mL" and may be diluted using the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution: 1:2

Specimen must be diluted before pretreatment.

1. 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.
2. 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 should be > 3.0 ng/mL before the dilution factor is applied.

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

Calibration

- Test Calibrators A-F in duplicate. The calibrators should be priority loaded.

  Only one pretreated sample of each ARCHITECT Sirolimus Calibrator is required to perform a calibration on the ARCHITECT iSystem. This provides adequate volume to run each calibrator in duplicate.

  A single sample of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- 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 or
  - 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. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.

Commercial controls are suitable for this purpose.

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 sample results are invalid and the samples must be retested. 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 Sirolimus assay belongs to method group 6.

RESULTS

Calculation

The ARCHITECT Sirolimus assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) 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 5.

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, and clinical impressions.
- If the sirolimus results are inconsistent with clinical evidence, additional testing is recommended.
- 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.
- 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.
- 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.[15]
- 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.[16, 17]

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 crossreactivity 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.[1]

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.[2] 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).

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.*

Sample	Instrument	Reagent Lot	n	Mean (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
Level 1	1	1	80	3.8	0.1	3.0	0.3	7.7
Level 1	2	2	80	3.6	0.1	3.6	0.2	6.8
Level 2	1	1	80	13.2	0.3	2.6	0.6	4.6
Level 2	2	2	80	12.1	0.3	2.4	0.7	5.5
Panel 1	1	1	80	8.1	0.2	2.6	0.4	5.2
Panel 1	2	2	80	7.8	0.2	3.0	0.4	5.2
Panel 2	1	1	80	15.3	0.3	2.3	0.7	4.8
Panel 2	2	2	80	14.7	0.4	3.0	0.8	5.4
Panel 3	1	1	80	5.0	0.2	3.1	0.2	4.9
Panel 3	2	2	80	4.8	0.2	3.5	0.3	5.7
Panel 4	1	1	80	11.2	0.4	4.0	0.5	4.9
Panel 4	2	2	80	10.7	0.3	2.6	0.6	5.3
Panel 5	1	1	80	21.9	0.5	2.3	0.8	3.7
Panel 5	2	2	80	20.9	0.7	3.2	1.1	5.2

* 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.*

Specimen	Endogenous Concentration (ng/mL)	Sirolimus Added (ng/mL)	Measured Concentration (ng/mL)	% Recovery[a]
A	0.0	3.0	2.7	90
B	0.0	3.0	2.9	97
C	0.0	3.0	2.9	97
D	0.0	3.0	2.7	90
E	0.0	3.0	2.5	83
				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 93%
A	0.0	27.0	23.2	86
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%

[a] $\% \text{ Recovery} = \frac{\text{Measured Concentration}}{\text{Endogenous Concentration} + \text{Sirolimus Added}} \times 100$

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

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 are summarized in the following table.*

Specimen	Dilution Factor	Observed Concentration (ng/mL)	Calculated Concentration[a] (ng/mL)	% Recovery[b]
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	29.6	106
	1:2.50	11.9	29.8	107
	1:5.00	6.6	33.0	119
	1:10.00	3.3	33.0	118
2	Undiluted	25.3	---	---
	1:1.11	23.6	26.2	104
	1:1.25	18.6	23.3	92
	1:1.43	17.9	25.6	101
	1:1.67	14.5	24.2	96
	1:2.50	9.9	24.8	98
	1:5.00	5.1	25.5	100
	1:10.00	2.7	27.0	109
3	Undiluted	29.9	---	---
	1:1.11	26.2	29.1	97
	1:1.25	21.6	27.0	90
	1:1.43	19.8	28.3	95
	1:1.67	17.4	29.1	97
	1:2.50	11.6	29.0	97
	1:5.00	6.3	31.5	105
	1:10.00	3.2	32.0	107
				Mean Recovery: 102

[a] Calculated Concentration = Observed Concentration x Dilution Factor

[b] $\% \text{ Recovery} = \frac{(\text{Observed Concentration x Dilution Factor})}{\text{Undiluted Observed Concentration}} \text{ x } 100$

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

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 two standard deviations above the ARCHITECT Sirolimus Calibrator A (0.0 ng/mL) was calculated to be 0.3 ng/mL* at the 95% level of confidence (based upon one study with n=24 runs, 10 replicates calibrator A and 4 replicates calibrator B per run).

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

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 sirolimus to achieve approximate concentrations from 0.1 to 5.3 ng/mL, were tested in replicates of 10, 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 Sirolimus assay value exhibiting a 20% CV was calculated to be 0.7 ng/mL.*

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

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.

Metabolite	Amount Added (ng/mL)	Mean Excess Concentration Detected (ng/mL, n-5)	% Cross Reactivity[a]
F2 (41-0-demethyl-hydroxyl-sirolimus)	10	0.87[b]	8.7
F3 (41-0-demethyl-hydroxyl-sirolimus; 7-0-demethyl-sirolimus)	3	0.12[b]	7.6
F4 (11-hydroxy-sirolimus)	10	3.7	36.8
F5 (41-0-demethyl-sirolimus)	10	2.0	20.3

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

[b] Concentration is below the reportable range of the ARCHITECT Sirolimus assay.

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

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.[19] 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%.*

Test Compound	Test Concentration	Test Compound	Test Concentration
Acetaminophen	200 µg/mL	Kanamycin B Sulfate	60 µg/mL
Acyclovir	15 µg/mL	Ketoconazole	50 µg/mL
Allopurinol	50 µg/mL	Labetalol	17.1 µg/mL
Amikacin*$2H_2O$	150 µg/mL	Lidocaine	60 µg/mL
Amphotericin B	5.8 µg/mL	Lovastatin	20 µg/mL
Apresoline	9.6 µg/mL	Minoxidil	60 µg/mL
Azathioprine	10 µg/mL	Mycophenolic Acid	25 µg/mL
Bromocriptine	1.5 µg/mL	Mycophenolic Acid Glucuronide	1800 µg/mL
Carbamazepine	120 µg/mL	N-Acetyl- Procainamide	100 µg/mL
Ceftriazone	840 µg/mL	Nadolol	1.2 µg/mL
Chloramphenicol	250 µg/mL	Nicardipine	0.5 µg/mL
Chloroquine	1.5 µg/mL	Penicillin G Na+	100 µg/mL
Cimetidine	100 µg/mL	Phenobarbital	150 µg/mL
Ciprofloxacin	7.4 µg/mL	Phenytoin	100 µg/mL
Clonidine	0.01 µg/mL	Prazosin	25 µg/mL
Colchicine	0.09 µg/mL	Prednisolone	100 µg/mL
Cortisone	1.2 µg/mL	Prednisone	100 µg/mL
Cyclosporine	3.2 µg/mL	Primidone	100 µg/mL
Digitoxin	0.08 µg/mL	Probucol	600 µg/mL
Digoxin	0.0048 µg/mL	Procainamide	100 µg/mL
Diltiazem	0.04 µg/mL	Propranolol	5 µg/mL

Test Compound	Test Concentration	Test Compound	Test Concentration
Disopyramide	30 µg/mL	Quinidine	50 µg/mL
Erythromycin	200 µg/mL	Ranitidine	200 µg/mL
Fluconazole	75 µg/mL	Rifampin	50 µg/mL
Flucytosine	240 µg/mL	Spectinomycin	100 µg/mL
5-Fluorocystosine	40 µg/mL	Tacrolimus	0.06 µg/mL
Furosemide	20 µg/mL	Ticlopidine	150 µg/mL
Ganciclovir	100 µg/mL	Tobramycin	20 µg/mL
Gemfibrozil	100 µg/mL	Trimethoprim	40 µg/mL
Gentamicin	120 µg/mL	Valproic Acid	144.2 µg/mL
Hydrocortisone	1.2 µg/mL	Vancomycin	60 µg/mL
Itraconazole	50 µg/mL	Verapamil	10 µg/mL
Kanamycin A Sulfate	60 µg/mL		

Potential Interfering Substance	Concentration
Triglycerides	1500 mg/dL
Hematocrit	$\leq$ 25%, $\geq$ 55%
Bilirubin	40 mg/dL
Total Protein	3 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

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

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[20] method. Data from this study are summarized in the following table.*

ARCHITECT Sirolimus vs IMx Sirolimus			
Number of Observations	Intercept (95% CI[a])	Slope (95% CI)	Correlation Coefficient
168	0.12 (-0.38 to 0.47)	1.05 (1.00 to 1.11)	0.94

[a] Confidence Interval (CI)

Specimen Range (ARCHITECT): 2.1 ng/mL to 29.7 ng/mL

Specimen Range (IMx): 1.8 ng/mL to 29.6 ng/mL

* Representative data; variables such as differences in sampling size and sample population may impact the correlation of the assay, therefore, 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[20] method. Data from this study are summarized in the following table.*

ARCHITECT Sirolimus vs LC/MS/MS			
Number of Observations	Intercept (95% CI)	Slope (95% CI)	Correlation Coefficient
167	-0.37 (-0.89 to 0.12)	1.18 (1.11 to 1.27)	0.91

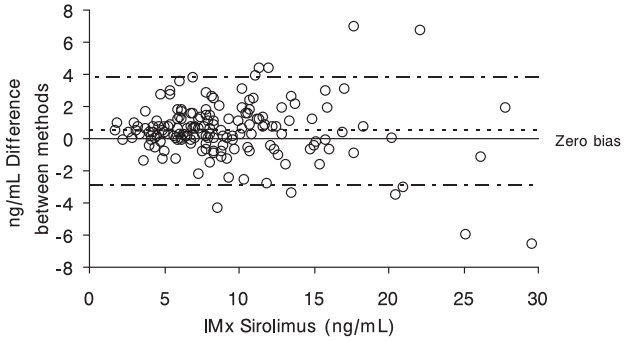
Specimen Range (ARCHITECT): 2.1 ng/mL to 29.7 ng/mL

Specimen Range (LC/MS/MS): 1.65 ng/mL to 29.1 ng/mL

* Representative data; variables such as differences in sampling size and sample population may impact the correlation of the assay, therefore, results in individual laboratories may vary from these data.

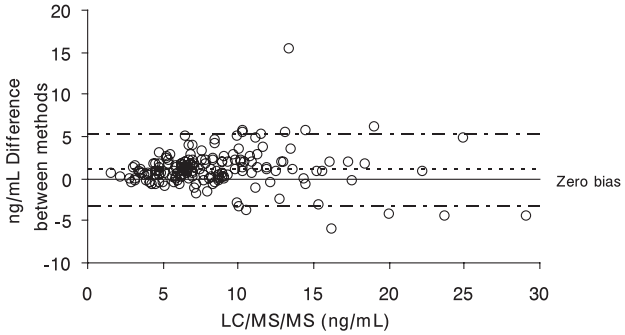
A bias analysis of the ARCHITECT Sirolimus vs. IMx Sirolimus assay was performed on the same 168 human whole blood EDTA samples in the range of 2 to 30 ng/mL. The following representative data are provided to aid in understanding the differences between these two assays. The average ng/mL difference 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
Bias from IMx Sirolimus



A bias analysis of the ARCHITECT Sirolimus vs. LC/MS/MS 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. LC/MS/MS assay in this study was 1.08 ng/mL. The 95% confidence interval of the ng/mL difference bias is 0.74 ng/mL to 1.42 ng/mL. Results of the study are summarized below.

ARCHITECT Sirolimus ng/mL Difference
Bias from LC/MS/MS



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

BIBLIOGRAPHY

1. MacDonald A, Scarola J, Burke JT, et al. Clinical pharmacokinetics and therapeutic drug monitoring of sirolimus. *Clin Ther* 2000;22(Suppl B):B101-B121.
2. Oberbauer R, Kreis H, Johnson RW, et al. Long-term improvement in renal function with sirolimus after early cyclosporine withdrawal in renal transplant recipients: 2-year results of the rapamune maintenance regimen study. Transplantation 2003;76(2):364-370.
3. Sehgal SN, Baker H, Vézina C. Rapamycin (AY-22,989), a new antifungal antibiotic: II. Fermentation, isolation and characterization. *J Antibiot* 1975;28(10):727-732.
4. Shaw LM, Kaplan B, Brayman KL. Introduction and overview - advances in therapeutic drug monitoring for immunosuppressants: a review of sirolimus. *Clin Ther* 2000;22(Suppl B):B1-B13.
5. Mahalati K, Kahan BD. Clinical pharmacokinetics of sirolimus. *Clin Pharmacokinet* 2001;40(8):573-585.
6. Rapamune [package insert]. Philadelphia, PA: Wyeth Laboratories; 2006
7. Zimmerman JJ, Kahan BD. Pharmacokinetics of sirolimus in stable renal transplant patients after multiple oral dose administration. *J Clin Pharmacol* 1997;37:405-415.
8. Holt DW, McKeown DA, Lee TD, et al. The relative proportions of sirolimus metabolites in blood using HPLC with mass-spectrometric detection. *Transplant Proc* 2004;36:3223-3225.
9. Salm P, Taylor PJ, Pillans PI. Analytical performance of microparticle enzyme immunoassay and HPLC-tandem mass spectrometry in the determination of sirolimus in whole blood. *Clin Chem* 1999;45(12):2278-2280.
10. Maleki S, Graves S, Becker S, et al. Therapeutic monitoring of sirolimus in human whole-blood samples by high-performance liquid chromatography. *Clin Ther* 2000;22(Suppl B):B25-B37.
11. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.

12. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
13. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
14. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.
15. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988;34(1):27-33.
16. Primus FJ, Kelley EA, Hansen HJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-264.
17. Schroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45(2):879-885.
18. National Committee for Clinical Laboratory Standards (NCCLS). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.* NCCLS Document EP5-A2. Wayne, PA: NCCLS; 2004.
19. Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition.* CLSI Document EP7-A2. Wayne, PA: CLSI; 2005.
20. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. *J Clin Chem Clin Biochem* 1983;21(11):709–720.

Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ASSAY DILUENT	Assay Diluent
CENTRIFUGE	Centrifuge
CENTRIFUGE TUBES	Centrifuge Tubes
CONJUGATE	Conjugate
CONTROL NO.	Control Number
EC REP	Authorized Representative in the European Community
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF USA	Product of USA
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer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