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| **Sirolimus** |
| **Purpose** | This procedure provides instructions for performing SIROLIMUS on the Abbott Alinity ci series chemistry analyzer. |
| **Principle** | This assay is a delayed one-step immunoassay for the quantitative determination of sirolimus in human whole blood using chemiluminescent microparticle immunoassay (CMIA) technology. Prior to the initiation of the automated Alinity i 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 Alinity i analyzer. Sample, anti-sirolimus coated paramagnetic microparticles, and assay diluent are combined and incubated. The sirolimus present in the sample binds to the anti-sirolimus coated microparticles. Sirolimus acridinium-labeled conjugate is added to create a reaction mixture. The sirolimus acridinium-labeled conjugate competes for the available binding sites on the anti-sirolimus coated paramagnetic microparticles. The reaction mixture is incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. 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 system optics.For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3.  |
| **Policy Statements** | This procedure applies to all personnel responsible for performing Sirolimus testing on the Abbott Alinity ci analyzer in Minneapolis laboratory. |
| **Clinical Significance** | 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 In this study, clinical outcomes of patients withdrawn from cyclosporine and maintained on sirolimus and corticosteroids compared favorably to patients continuing on the triple-drug immunosuppressive regimen.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 FKbinding 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.6 Ascending dose studies (range 0.5 - 6.5 mg/m2/12 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 (r2 = 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 andcorticosteroids, there was a 4.5 fold difference in apparent meandrug 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 bemonitored no less than 5 - 7 days after a dosage change. Oncea day dosing is recommended in adult renal transplant patients.A loading dose (3 times the maintenance dose) can be used toachieve near steady-state blood concentrations rapidly.1 Variationsin apparent drug clearance and oral bioavailability result in a widerange of sirolimus trough values among patients receiving identicaldoses.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 ofpatients 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 the parent drug and the methods that detect both parent drug and its metabolites. |
| **Instrument** | **PRIMARY METHOD: Abbott Alinity ci****SECONDARY (BACKUP) METHOD: Mayo Medical Laboratories (Sunquest Test Code TACR)** |
| **Sunquest Test Code** | * SIRC
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| **Specimen** | **Sample type:** EDTA whole blood**Minimum volume:** 300 uL whole blood EDTA**Stability: 2-8°C for 6 days, -10°C for 6 months****Rejection criteria:** Unlabeled specimens, incorrect sample type, cadaver specimens or any other body fluids, obvious microbial contamination.**Preparation:** 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 |
| **Reagents** |

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity I Sirolimus Reagent | 09P4120 | **Store at:** 2 – 8 °C**Unopened/Opened:** Manufacturer expiration date.**On-board:** 30 Days |
| Alinity I Sirolimus Calibrator | 09P4101 | **Store at:**  2 – 8 °C**To Use**: Gently mix at least 10 times immediately prior to aliquoting**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, until manufacturer expiration date |
| Alinity i Sirolimus Whole Blood Precipitation Reagent | 09P4140 | Store at Room Temperature. Stable until expiration date when stored tightly capped. |
| Transplant Pretreatment Tubes | 01P06 | Store at Room Temperature. Stable until package expiration date. Use one tube per specimen; use only once. |
| BioRad Whole Blood Immunosuppressant QC | Level 1 - 12000404Level 2 - 12000405Level 3 - 12000406 | **Unopened storage:** <-20°C**To Use:** Thaw at room temperature for 30 minutes prior to use. Invert and swirl gently. **Once Opened, Store:** 2-8°C**Stability:** 10 Days |

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| **Calibration and Analytical Measuring Range (AMR)** |

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| Analytical Measuring Range: | 2-30 ng/mL |
| Reference Material: | Abbott Alinity I Sirolimus Calibrators (1L77-01) |
| Suggested Calibration Levels | A – 0.0B – 3.0C – 6.0D – 12.0E – 20.0F – 30.0 |
| Verification Scheme: | n=6 |
| Verification Frequency: | * For each new lot of reagent
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
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| Analytical Measuring Range Verification | Verification of AMR is accomplished with each calibration.Cal Verification and AMR verification are performed at least once every six (6) months. |
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| **Manual Pre-Treatment Procedure** |

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| 1 | Label the appropriate number of bullet tubes (using a foot label for patient samples, and a sharpie for QC/Calibrators). |
| 2 | Invert the sample (patient, control and calibrator) 10 times **minimum** until sample is well mixed. |
| 3 | Pipette exactly 150.0 µL of sample into appropriately labeled bullet tube, using a new tip with each sample. Do not wipe pipette tip. Do not over-aspirate. Do not pre-wet the tip as it may add imprecision to the assay.  |
| **Finish pipetting all patient/blood/QC samples before moving on to the next step** |
| 4 | Add exactly 300.0 µL of Architect Sirolimus Whole Blood Precipitation Reagent. |
| 5 | Cap and vortex immediately for no less than 10 seconds before moving onto the next bullet tube (improper vortexing time will lead to erroneous results). Ensure no unmixed portion is left at the bottom of the tube after vortexing.  |
| 6 | Place in 42 degrees C heat block and immediately move onto the next tube.  |
| **Repeat steps 4-6 on each tube before moving on to the next step. Incubate at 42 degrees C for exactly 10 minutes.**  |
| 7 | After 10 minutes, IMMEDIATELY Centrifuge all samples (controls/calibrators/patients) for 4 minutes at 10,000g. While tubes are spinning, begin to label each Transplant Pretreatment Tube with a barcoded patient label. Also, record the temperature of the heatblock on the log if not already recorded. The block must be 42 degrees +/- 1 degree. |
| 8 | Remove each tube from the centrifuge and confirm the presence of a well formed pellet and clear supernatant. If this is not present, you must start over from step 1. |
| 9 | Uncap each tube and pour off the supernatant into the appropriately labeled Transplant Pretreatment Tube. Be sure not to disturb the pellet. Replace the pink cap to the Transplant Pretreatment Tube. You may discard the tube with the pellet into biohazard trash after decanting the supernatant.  |
| 10 | Vortex the Transplant Pretreatment Tube for no less than 5 seconds, and up to 10 seconds, then place in an Alinity sample rack. |
| 11 | Place the sample rack in a Priority lane (section with a blue light) on the Alinity ci. |

**Important Notes Regarding Pretreatment Procedure**1. All Pretreated samples (patients/controls/calibrators) must be tested within **30 minutes** of being decanted into the Transplant Pretreatment Tubes and placed onto the Alinity ci. Controls must be tested with each run.
2. All Sirolimus samples must be priority loaded onto the analyzer (blue light at carrier position.)
3. If for any reason a specimen fails to sample on the analyzer, the entire process must be repeated at the next available run time from step 1, including QC.
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| **Risk and Safety** | Sirolimus Precipitation Reagent contains methanol, zinc sulfate and ethylene glycol. Dispose of in the properly labeled methanol satellite container.  |
| **Quality Control** | BioRad Liquichek Whole Blood Immunosuppressant QC (WBIS)**Frequency:** Three levels with each Sirolimus run.**Stability Unopened:** Until expiration date @ <-20°C**Stability After Opening:** 10 days @ 2-8°C.**Preparation**: Control materials are ready for use. Thaw one bottle of each level at room temperature for 20 minutes prior to use. Mix well by gentle inversion at least 10 times immediately prior to pipetting.**Acceptable ranges:** • Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules. • New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot. • Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte. • Acceptable ranges are current in Unity Real Time only. Quality Control results must be rejected in Sunquest when the results cross the interface. • In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.• Do not load or release patients until QC is acceptable in Unity Real Time. |
| **Interferences** | * 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 that employ mouse monoclonal antibodies.
* 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 results may be observed. Additional information may be required for diagnosis. Immunoassays are nonspecific and cross react with metabolites.
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| **Reference Range** | 4 - 20 ng/mLNote: Therapeutic range applies to trough specimen drawn immediately prior to a.m. dose. Blood drawn at other times will yield higher results.Most individuals display optimal response to sirolimus with trough whole blood levels 4 ng/mL to 20 ng/mL. Preferred therapeutic ranges may vary by transplant type, protocol, and co-medications. |
| **Critical Values** | None Specified |
| **Limitations** | * 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.
* 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 SPECIFIC PERFORMANCE CHARACTERISTICS, Specificity section of the package insert for estimates of cross-reactivity of ALINITY Sirolimus to some metabolites of sirolimus.
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| Dilutions | Samples >30.0 may be diluted 1:2 with Calibrator A.  |
| **Result Reporting** | * Results <2.0 ng/mL will be reported as <2.0 ng/mL
* Results >30.0 ng/mL may be manually diluted 1:2 and reported as the numerical value if below 60.0. Results >60.0 after dilution will be reported as >60.0.
* Occasionally, the provider may request results between 1.0 and 2.0 ng/mL. Send these samples to Mayo for analysis by Mass Spectrometry.
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| **Specimen Storage** | Pretreated samples will be discarded once the samples are resulted. To repeat testing, sample must be undergo the entire precipitation process. The original whole blood samples will be stored in the freezer for 7 days. |
| **References** | 1. ABBOTT ARCHITECT Sirolimus package insert. Abbott Laboratories Diagnostics Division Abbott Park, IL 60064. Revised August 2019.
2. ABBOTT ARCHITECT Sirolimus Calibrator package insert. Abbott Laboratories Diagnostics Division Abbott Park, IL 60064. Revised May 2019.
3. Bio-Rad Liquichek Whole Blood Immunosuppressant Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618. Revised August 2018.
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| **Historical Record** |

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| **Version** | **Written/Reviewed by:** | **Effective date** | **Summary of Revisions** |
| 1 | Erin Bartos | 10/28/2020 | New Procedure for Alinity i |
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