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| **Tacrolimus** |
| **Purpose** | This procedure provides instructions for performing TACROLIMUS on the Abbott Alinity ci and Abbott Architect. |
| **Principle** | The Abbott Tacrolimus assay is a delayed one-step chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of tacrolimus in human whole blood on the Alinity ci with flexible assay protocols, referred to as Chemiflex. The tacrolimus assay is to be used as an aid in the management of liver and kidney allograft patients receiving tacrolimus drug therapy.Prior to the initiation of the automated Alinity or ARCHITECT sequence, a manual pretreatment step is performed in which the whole blood sample is extracted with a precipitation reagent and centrifuged. The supernatant is decanted into a Transplant Pretreatment Tube, which is placed onto the Alinity ci.1. Sample, assay diluent, and anti-tacrolimus coated paramagnetic microparticles are combined to create a reaction mixture. The tacrolimus present in the sample binds to the anti-tacrolimus coated microparticles.
2. After a delay, tacrolimus acridinium-labeled conjugate is added to the reaction mixture. The tacrolimus on the acridinium-labeled conjugate competes for the available binding sites on the microparticles.
3. After further incubation and washing, 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 tacrolimus in the sample and the RLUs detected by the ARCHITECT or Alinity iSystem optics.

For additional information on system and assay technology, refer to the Operations Manuals, Section 3. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing Tacrolimus testing on the Abbott Alinity ci and Architect i1000SR. Tacrolimus reagent shall be loaded on the Architect i1000SR in event of Alinity ci downtime. Reagent will be calibrated and quality control completed with each patient run as directed by the manufacturer. |
| **Clinical Significance** | Tacrolimus is an immunosuppressive drug discovered in 1984 by the Fujisawa Pharmaceutical Co., Ltd. It has been shown to be effective for the treatment of organ rejection following transplantation. The results of clinical trials with liver and kidney have been published. Clinical studies are continuing for a variety of indications.Pharmacokinetic studies have also indicated that whole blood rather than plasma may serve as the more appropriate medium to describe the pharmacokinetic characteristics of tacrolimus. Tacrolimus is bound to proteins, mainly albumin and α-1-acid glycoprotein, and is highly bound to erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors such as hematocrit, temperature of separation of plasma, drug concentration, and plasma protein concentration.Tacrolimus binds to a family of proteins termed FK506 (tacrolimus) binding proteins (FKBPs). The formation of a larger pentameric complex comprised of FKBP, tacrolimus, calmodulin and calcineurins A and B results in the inhibition of the phosphatase activity of calcineurin. The action of transcription factors requiring dephosphorylation for transport to the cell nucleus are thus inhibited leading to blockage of T-cell proliferation and function.The use of tacrolimus is associated with serious toxic side effects, primarily nephrotoxicity. At the present time it is not clear whether the nephrotoxicity of tacrolimus is the result of parent drug, metabolites, or a combination of both. Other adverse side effects include neurotoxicity, hypertension, insomnia, and nausea. |
| **Instrument** | **PRIMARY METHOD: Abbott Alinity ci****SECONDARY (BACKUP) METHOD: Abbott Architect i1000 SR** |
| **Sunquest Test Code** | * **TACL**
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| **Specimen** | **Sample type:** EDTA whole blood**Minimum volume:** 250 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*** |
| Abbott Alinity I Tacrolimus Reagent | 09P4220 | **Store at:** 2 – 8 °C**Unopened/Opened:** Manufacturer expiration date.**On-board:** 30 Days |
| Abbott Alinity i Tacrolimus Calibrator | 09P4201 | **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 when stored tightly capped and minimal time at room temperature |
| Abbott Architect Tacrolimus Reagent | 1L77-25 | **Store at**: 2 – 8 °C**Unopened/Opened:** Manufacturer expiration date.**On-board**: 30 Days |
| Abbott Architect Tacrolimus Calibrators | 1L77-01 | **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 when stored tightly capped and minimal time at room temperature |
| Tacrolimus Precipitation Reagent | 09P4240 | **Store at:** 15 - 25°C**Unopened**: Manufacturer expiration date.**Opened:** Store at 2 – 8 °C, until manufacturer expiration date**Contains Methanol. Pour used reagent into Methanol satellite container** |
| BioRad Whole Blood Immunosuppressant QC | Level 1 - 12000404Level 2 - 12000405Level 3 - 12000406 | **Unopened storage:** <-20°C**To Use:** Thaw at room temperature for 30 minute prior to first use. Mix by gentle inversion at least 10 times immediately prior to use **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 Architect Tacrolimus CalibratorsAbbott Alinity Tacrolimus Calibrators |
| 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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| **Pre-Treatment Procedure** | Architect and Alinity:

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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 200 µL of sample into a bullet tube. |
| **Finish pipetting all patient/blood/QC samples before moving on to the next step** |
| 4 | Add exactly 200 µL of Architect Tacrolimus 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). |
| 6 | Place in centrifuge and move onto the next tube.  |
| **Repeat steps 4-6 on each tube before moving on to the next step** |
| 7 | 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. |
| 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.  |
| 11 | Place in a Priority lane (section with a blue light) on the Architect i1000SR or Alinity i. |

**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 Architect i1000SR or Alinity i. Controls must be tested with each run.
2. All Tacrolimus 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** | Tacrolimus Precipitation Reagent contains methanol, zinc sulfate and ethylene glycol. Dispose of in the properly labeled methanol satellite container. Contains methylisothiazolones and sodium azide. Avoid contact with skin and eyes. Causes serious eye irritation. Wear gloves. Contact with acids liberates very toxic gas. Recap and dispose of in appropriate Hazardous Waste Container. |
| **Quality Control** | BioRad Liquichek Whole Blood Immunosuppressant QC Levels 1,2,3 (WBIS)**Frequency:** Three levels with each Tacrolimus 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 30 minutes prior to use. Mix well by gentle inversion at least 10 times immediately prior to pipetting.**Acceptable ranges:** * Ranges are current in Unity Real Time only.
* If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established.
* Do not release patient results until QC is reviewed in Unity RealTime and the cause of any deviations have been identified, corrected, documented, and acceptable QC results are generated
* When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range

When a new lot of unassayed control is received, verify new ranges by running the new lot in parallel with the current lot for 20 consecutive days whenever possible. Ranges will be developed by comparing to peers by the Technical Specialist.  |
| **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** | None Specified- Acceptable tacrolimus results are patient-specific. |
| **Critical Values** | None Specified |
| **Limitations** | * Immunoassays are nonspecific and cross react with metabolites. When elimination of tacrolimus is impaired (e.g. during cholestasis), tacrolimus metabolites may accumulate. The immunoassay may overestimate the concentration of tacrolimus. In such cases, the use of a specific assay (e.g. Liquid Chromatography Mass Spectrometry/Mass Spectrometry [LC/MS/MS]) could be considered.
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| Dilutions | Do not dilute. |
| **Result Reporting** | * Results <2.0 ng/mL will be reported as <2.0 ng/mL
* Results >30.0 ng/mL will be flagged and reported as >30.0 ng/mL. Do not dilute.
* Occasionally, the provider may request results between 1.0 and 2.0 ng/mL. In this case, reorder the test with Mayo test code TACR and send for analysis by Mass Spectrometry to Mayo Medical Laboratories.
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| **Specimen Storage** | Pretreated samples will be discarded once the samples are resulted. To repeat testing, or in event quality controls fail, 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 Tacrolimus package insert. Abbott Laboratories Diagnostics Division Abbott Park, IL 60064. Revised September 2015.
2. ABBOTT ARCHITECT Tacrolimus Calibrator package insert. Abbott Laboratories Diagnostics Division Abbott Park, IL 60064. Revised October 2014.
3. ABBOTT Alinity i Tacrolimus package insert. Abbott Laboratories Diagnostics Division Abbott Park, IL 60064. Revised August 2019
4. ABBOTT Alinity i Tacrolimus Calibrators package insert. Abbott Laboratories Diagnostics Division Abbott Park, IL 60064. Revised May 2019
5. 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 | E. Bartos, K. Brown, S. Gripentrog | 12/17/2018 | New Procedure |
| 2 | Erin Bartos | October 28, 2020 | Added Alinity i |
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