**Procedure Title:** Cyclosporine – Abbott Architect

**Document Section:** CHEMISTRY  
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**Date Authored:** JUNE 2009

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I. PURPOSE

The ARCHITECT Cyclosporine assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of cyclosporine in human whole blood on the ARCHITECT i System. The ARCHITECT Cyclosporine assay is used as an aid in the management of heart, liver and kidney transplant patients receiving cyclosporine therapy.

SUMMARY AND EXPLANATION OF TEST

Cyclosporine is a cyclic undecapeptide of fungal origin and a potent immunosuppressant. It is used as a primary agent during immunosuppressive therapy for solid organ transplants. Immunosuppression is thought to be the result of impairment of T-cell receptor transcription of the IL-2 gene. Cyclosporine therapy has greatly improved organ transplant survival of heart, liver and kidney transplants.

Cyclosporine may be administered by IV or orally. Absorption from the gastrointestinal tract is variable, unpredictable, and incomplete. Bioavailability increases during treatment so oral doses must be gradually reduced in order to maintain a constant cyclosporine concentration in the blood. Assessment of cyclosporine concentrations in blood aids in adjusting patients' dosage and avoids cyclosporine underdosage inefficacy or overdosage toxicity. Cyclosporine is eliminated almost completely by hepatic metabolism, cytochrome P-450 enzymes being responsible for the biotransformation of cyclosporine and its metabolites. More than thirty metabolites have been identified. Preliminary data indicate cyclosporine metabolites are less immunosuppressive and less toxic than their parent compound.

Many drugs affect cyclosporine blood concentrations. These drugs alter cyclosporine blood concentration by inducing drug metabolism, interfering with drug metabolism, or affecting drug absorption. Such interactions between cyclosporine and danazol, diltiazem, erythromycin, fluconazole, itraconazole, ketoconazole, metoclopramide, nicardipine, verapamil, carbamazepine, phenobarbital, phenytoin, rifampicin, and cotrimoxazole are well documented.

The use of cyclosporine is associated with serious toxic side effects, primarily nephrotoxicity and hepatotoxicity. Other adverse effects include diarrhea, gum hyperplasia, nausea, vomiting, hirsutism, tremor, and hypertension. Some studies have shown the benefits of monitoring cyclosporine concentrations, including a reduction in the incidence of biopsy proven acute rejection.

II. POLICY/SCOPE

This procedure is intended for use by licensed CLS staff in the China Basin Chemistry section.

III. TEST AVAILABILITY

Test performed daily. Samples received in the lab by 12 noon will be resulted the same day before 1600.

IV. SPECIMEN REQUIREMENTS

SPECIMEN TYPES

1. Only human whole blood specimens collected in EDTA tubes may be used with the ARCHITECT Cyclosporine assay.

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

3. Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient samples.

4. The ARCHITECT i System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen type is used in the ARCHITECT Cyclosporine assay.
STORAGE

1. Specimens collected in EDTA tubes may be stored up to 7 days refrigerated at 2-8 degrees C. prior to being tested. If testing will be delayed more than 7 days, store frozen (-10 degrees C. or colder). Specimens must be mixed thoroughly after thawing to ensure consistency of the results.

2. Avoid multiple freeze/thaw cycles.

3. Discard any remaining pretreated samples after testing is complete. ARCHITECT Cyclosporine tests cannot be reordered. A retest requires that the Manual Pretreatment Procedure in the PROCEDURE section be repeated.

V. EQUIPMENT, REAGENTS AND SUPPLIES

TEST INSTRUMENT: Abbott ARCHITECT System

MATERIALS PROVIDED

1. 1L75 ARCHITECT Cyclosporine Reagent Kit
2. 1L75-55 ARCHITECT Cyclosporine Whole Blood Precipitation Reagent Kit
3. 9527-40 XSYSTEMS CENTRIFUGE TUBES
4. 1P06-01 Transplant Pretreatment Tubes

MATERIALS REQUIRED BUT NOT PROVIDED

1. ARCHITECT i System
2. 3K50 ARCHITECT i ASSAY CD-ROM - US - Addition A
3. 3K52 ARCHITECT i ASSAY CD-ROM - WW (excluding US) - Addition A
4. 1L75-01 ARCHITECT Cyclosporine Calibrators
5. BioRad Lyphochek Immunosuppressant controls levels 1 and 4
6. Vortex Mixer
7. 9527-26 XSYSTEMS CENTRIFUGE
8. ARCHITECT i PRE-TRIGGER SOLUTION
9. ARCHITECT i TRIGGER SOLUTION
10. ARCHITECT i WASH BUFFER
11. ARCHITECT i REACTION VESSELS
12. ARCHITECT i SEPTUM
13. ARCHITECT i REPLACEMENT CAPS
14. Precision Micropipettes
15. Pipette tips
16. 9528-02 XSYSTEMS PRECISION DISPENSER, or equivalent
17. 2.5 mL Combitips, or equivalent, for PRECISION DISPENSER

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

REAGENTS

REAGENT KIT, 100 TESTS

ARCHITECT CYCLOSPORINE REAGENT KIT (1L75)

1. MICROPARTICLES: 1 Bottle (8.0 mL) Anti-cyclosporine (mouse, monoclonal) coated microparticles in MOPS buffer with protein (bovine) stabilizer. Preservatives: sodium azide and ProClin 950.

2. CONJUGATE: 1 Bottle (12.0 mL) Cyclosporine acridinium-labeled conjugate in citrate buffer with detergent. Preservative: ProClin 300.

3. ASSAY DILUENT: 1 Bottle (10.0 mL) Assay Diluent containing MES buffer and NaCl. Preservative: ProClin 300.

OTHER REAGENTS

ARCHITECT i PRE-TRIGGER SOLUTION

PRE-TRIGGER SOLUTION: Pre-trigger solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT i TRIGGER SOLUTION

TRIGGER SOLUTION: Trigger solution containing 0.35 N sodium hydroxide.

ARCHITECT i WASH BUFFER


STORAGE INSTRUCTIONS

1. The ARCHITECT Cyclosporine Reagent Kit must be stored at 2-8 degrees C. in an upright position and may be used immediately after removal from 2-8 degrees C. storage.

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

3. The ARCHITECT Cyclosporine Reagent Kit may be stored on board the ARCHITECT i System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. Recalibration may be required to obtain maximum onboard reagent stability. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

4. Reagents may be stored on or off the ARCHITECT i System. If reagents are removed from the system, store them at 2-8 degrees C. (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.

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.

VI. WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use.

SAFETY PRECAUTIONS

CAUTION

This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

1. The microparticles, conjugate and assay diluent contain methylisothiazolones, which are components of ProClin, and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

   R43    May cause sensitization by skin contact.
   S24    Avoid contact with skin.
   S35    This material and its container must be disposed of in a safe way.
   S37    Wear suitable gloves.
   S46    If swallowed, seek medical advice immediately and show this container or label.

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

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

HANDLING PRECAUTIONS

1. Do not use reagent kits beyond the expiration date.

2. Do not pool reagents within a kit or between reagent kits.

3. Before loading the ARCHITECT Cyclosporine Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the PROCEDURE, Assay Procedure section of this package insert.

4. Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.

5. To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.

   a. Once a septum has been placed on the reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.

   b. Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.
6. For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

VII. CALIBRATION/ CALIBRATION VERIFICATION

1. To perform an ARCHITECT Cyclosporine calibration, test calibrators A, B, C, D, E, and F in replicates of two. Only one pretreated sample of each ARCHITECT Cyclosporine Calibrator is required to perform a calibration on the ARCHITECT i System. This provides adequate volume to run each calibrator in duplicate. A single pretreated sample of each cyclosporine control must be tested to evaluate the assay calibration. Ensure that assay control values are within established ranges.

2. Calibration Range: 0 - 1500 ng/mL.

3. Once an ARCHITECT Cyclosporine calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
   a. A reagent kit with a new lot number is used.
   b. Controls are out of range.

4. For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

VIII. QUALITY CONTROL

BioRad Lyphochek Whole Blood Immunosuppressant controls Levels 1 and 4.

Reconstitution instructions.
Using a volumetric pipet, reconstitute each vial with 2.0 mL of deionized water. Replace the stopper and allow the control to stand for at least 60 minutes, swirling occasionally. Before sampling, gently swirl the contents until homogeneous with no visible signs of particulate. Stable for 14 days at 2-8°C

Storage and stability.
This product is stable until the expiration date when stored at 2-8°C. Once the control is reconstituted, all analytes will be stable for 14 days when stored at 2-8°C.

BioRad Immunosuppressant controls levels 1 and 4 are run each day of testing

IX. PROCEDURE

ASSAY PROCEDURE

1. Before loading the ARCHITECT Cyclosporine Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
   a. Invert the microparticle bottle 30 times by hand.
   b. Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue inverting the bottle until the microparticles have been completely resuspended.
   c. If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.
   d. Once the microparticles have been resuspended, place a septum on the bottle. For
instructions on placing septums on bottles, refer to the Handling Precautions section of this package insert.

2. Load the ARCHITECT Cyclosporine Reagent Kit on the ARCHITECT i System.
   a. Verify that all necessary reagents are present.
   b. Ensure that septums are present on all reagent bottles.

3. Order calibration, if necessary.

   For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.

4. Order tests.

   For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.

5. No more than four replicates may be sampled from the same Transplant Pretreatment Tube.
   a. All pretreated samples (specimens, calibrators or controls) must be tested within 3 hours of being decanted into the Transplant Pretreatment Tubes and placed on the ARCHITECT i System.
   b. With the Transplant Pretreatment Tube, use the sample gauge to ensure sufficient patient specimen is present for the ARCHITECT Cyclosporine assay.

6. Prepare calibrators and controls.

   Refer to the Manual Pretreatment Procedure in the PROCEDURE section.

7. Load pretreated samples.

   For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5

8. Press RUN.

9. For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.

10. For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. When a laboratory requires more frequent maintenance, follow those procedures.

SPECIMEN DILUTION PROCEDURES

Specimens with a cyclosporine concentration of > 1500.0 ng/mL will be flagged as "> 1500.0 ng/mL" and should be diluted with the Manual Dilution Procedure.

1. Manual dilutions should be performed as follows:
   a. The suggested dilution for the ARCHITECT Cyclosporine assay is 1:4.
   b. Specimen must be diluted before pretreatment.
   c. Add 100 uL of the patient specimen to 300 uL of ARCHITECT Cyclosporine Calibrator A, mix well then proceed with the Manual Pretreatment Procedure in the PROCEDURE section.
d. 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 dilution should be performed so that the diluted result (before the dilution factor is applied) reads greater than 200.0 ng/mL.

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

MANUAL PRETREATMENT PROCEDURE

1. Mix each sample (specimen, calibrator, or control) thoroughly by slow inversion of the container 5-10 times. Visual inspection is recommended to assure the specimen is adequately mixed.

2. Precision pipette 200 μL of each sample into a labeled XSYSTEMS Centrifuge Tube immediately after mixing. Use a different centrifuge tube for each sample.

NOTE:

A new pipette tip must be used each time 200 μL is aspirated. Do not wipe pipette tip. Do not over aspirate. Do not reuse pipette tips between replicates.

3a. Set a Precision Dispenser (Repeater Pipette) to dispense 100 μL. Fill the dispenser with a sufficient volume of the ARCHITECT Cyclosporine Whole Blood Solubilization Reagent from the small orange-labeled bottle. **NOTE: Do not process/dispense more than 4 tubes at a time.**

   Purge air bubbles from the dispenser by dispensing a small amount of the solubilization reagent into a suitable waste container.

3b. Add 100 μL of ARCHITECT Cyclosporine Whole Blood Solubilization Reagent to the contents of each centrifuge tube (no more than 4 tubes at a time) with the end of the dispensing syringe tip touching the wall of the centrifuge tube.

4a. Set a Precision Dispenser (Repeater Pipette) to dispense 400 μL. Fill the dispenser with a sufficient volume of the ARCHITECT Cyclosporine Whole Blood Precipitation Reagent from the large orange-labeled bottle. **NOTE: Do not process/dispense more than 4 tubes at a time.**

   Purge air bubbles from the dispenser by dispensing a small amount of the precipitation reagent into a suitable waste container.

NOTE:

The ARCHITECT Cyclosporine Whole Blood Precipitation Reagent is highly volatile. Keep tightly closed when not in use to prevent evaporation.

4b. Add 400 μL of ARCHITECT Cyclosporine Whole Blood Precipitation Reagent to the contents of each centrifuge tube (no more than 4 tubes at a time) with the end of the dispensing syringe tip touching the wall of the centrifuge tube.

4c. Cap all of the centrifuge tubes and vortex after adding the ARCHITECT Cyclosporine Whole Blood Precipitation Reagent to all of the centrifuge tubes.

4d. Vortex vigorously for 5-10 seconds. Use the maximum vortex setting.

NOTE:

Visual inspection is required to ensure that the mixture of the sample with the solubilization and precipitation reagents 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. Not all vortex mixers may provide adequate mixing.

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

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

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

WARNING:
Do not disturb the pellet. Do not pipette the supernatant as this will help insure that the pellet is not disturbed.

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

WARNING:
Only Transplant Pretreatment Tubes (LN 1P06-01) are acceptable when pretreating cyclosporine samples for use on the ARCHITECT i System. Reliability of other ARCHITECT assay results may be affected if the Transplant Pretreatment Tubes are not used with the ARCHITECT Cyclosporine assay.

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

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

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

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

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

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

X. RESULTING/REPORTABLE RANGE

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

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.

ANALYTICAL MEASUREMENT RANGE (REPORTABLE RANGE)

The measurement range for the ARCHITECT Cyclosporine assay is 25 mcg/L (minimum reportable value based on Analytical Sensitivity) to 1500.0 mcg/L.

XI. EXPECTED VALUES
50-500 mcg/L.

No firm therapeutic range exists for cyclosporine in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of cyclosporine, co-administration of other immunosuppressants, type of transplant, time post-transplant and a number of other factors contribute to different requirements for optimal blood levels of cyclosporine. Therefore, individual cyclosporine 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 user must establish his or her own ranges based on clinical experience.

Therapeutic 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 assay methods and cross-reactivity with metabolites, nor should correction factors be applied. Therefore, consistent use of one assay for individual patients is recommended.

XII. LIMITATIONS OF PROCEDURE

1. Results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.

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

3. The concentration of cyclosporine in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

4. Immunoassays are nonspecific and cross react with metabolites. When elimination of cyclosporine is impaired (e.g. during cholestasis), cyclosporine metabolites may accumulate. The reported concentration of cyclosporine may be affected. In such cases, the use of a specific assay (e.g. Liquid Chromatography Mass Spectrometry/Mass Spectrometry [LC/MS/MS]) could be considered. Refer to the SPECIFICITY section for estimates of cross-reactivity of ARCHITECT Cyclosporine to some metabolites of cyclosporine.

5. 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 Cyclosporine) that employ mouse monoclonal antibodies.

6. 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.

XIII. SPECIFICITY/INTERFERENCES

INTERERECE

The ARCHITECT Cyclosporine assay is designed to have a mean recovery of 100 +/- 10% in the presence of the pharmaceutical substances, potentially interfering endogenous substances and potentially interfering clinical conditions at the levels below. A study based on guidance from the CLSI document EP7-A2 was performed for the ARCHITECT Cyclosporine assay.

POTENTIALLY INTERFERING PHARMACEUTICAL COMPOUNDS

Whole blood specimens spiked with cyclosporine targeting concentrations of 80 ng/mL and 800 ng/mL were supplemented with the following potentially interfering pharmaceutical compounds. The mean recoveries for the following compounds tested ranged from 90% to 109%.*
### Test Compound vs. Test Conc.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Test Conc.</th>
<th>Test Compound</th>
<th>Test Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>20 mg/dL</td>
<td>Hydrocortisone</td>
<td>1.2 µg/mL</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>3.2 µg/mL</td>
<td>Itraconazole</td>
<td>20 µg/mL</td>
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<tr>
<td>Allopurinol</td>
<td>5 mg/dL</td>
<td>Kanamycin A Sulfate</td>
<td>6 mg/dL</td>
</tr>
<tr>
<td>Amikacin*2H₂O</td>
<td>15 mg/dL</td>
<td>Ketoconazole</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>5.8 µg/mL</td>
<td>Labetalol</td>
<td>17.1 µg/mL</td>
</tr>
<tr>
<td>Apresoline</td>
<td>100 µg/mL</td>
<td>Lovastatin</td>
<td>20 µg/mL</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>1 mg/dL</td>
<td>Minoxidil</td>
<td>60 µg/mL</td>
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<tr>
<td>Bromocriptine</td>
<td>8 µg/mL</td>
<td>N-Acetyl-Procainamide</td>
<td>12 mg/dL</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>12 mg/dL</td>
<td>Nadolol</td>
<td>1.2 µg/mL</td>
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<tr>
<td>Cephalosporine</td>
<td>100 µg/mL</td>
<td>Nicardipine</td>
<td>0.5 µg/mL</td>
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<tr>
<td>Chloramphenicol</td>
<td>25 mg/dL</td>
<td>Pencillin G Na⁺</td>
<td>100 µg/mL</td>
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<tr>
<td>Chloroquine</td>
<td>1.5 µg/mL</td>
<td>Phenytoin</td>
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<tr>
<td>Cinetidine</td>
<td>10 mg/dL</td>
<td>Prazosin</td>
<td>25 µg/mL</td>
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<tr>
<td>Ciprofloxacin</td>
<td>7.4 µg/mL</td>
<td>Prednisolone</td>
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<tr>
<td>Clonidine</td>
<td>0.01 µg/mL</td>
<td>Prednisone</td>
<td>100 µg/mL</td>
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<tr>
<td>Colchicine</td>
<td>0.09 µg/mL</td>
<td>Primidone</td>
<td>10 mg/dL</td>
</tr>
<tr>
<td>Cortisone</td>
<td>1.2 µg/mL</td>
<td>Pro布colu</td>
<td>600 µg/mL</td>
</tr>
<tr>
<td>Digoxin</td>
<td>80 ng/mL</td>
<td>Procainamidene</td>
<td>10 mg/dL</td>
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<tr>
<td>Digoxin</td>
<td>4.8 ng/mL</td>
<td>Propranolol</td>
<td>0.5 mg/dL</td>
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<tr>
<td>Diltiazem</td>
<td>60 µg/mL</td>
<td>Quinidine</td>
<td>5 mg/dL</td>
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<tr>
<td>Disopyramide</td>
<td>3 mg/mL</td>
<td>Ranitidine</td>
<td>20 mg/dL</td>
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<tr>
<td>Erythromycin</td>
<td>20 mg/dL</td>
<td>Rifampin</td>
<td>5 mg/dL</td>
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<tr>
<td>Fluconazole</td>
<td>30 µg/mL</td>
<td>Sirolimus</td>
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<tr>
<td>Flucytosine</td>
<td>40 µg/mL</td>
<td>Tacrolimus</td>
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</tr>
<tr>
<td>Furosemide</td>
<td>2 mg/dL</td>
<td>Ticlopidine</td>
<td>150 µg/mL</td>
</tr>
<tr>
<td>Ganciclovir</td>
<td>1000 µg/mL</td>
<td>Tobramycin</td>
<td>2 mg/dL</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>100 µg/mL</td>
<td>Trimethoprim</td>
<td>40 µg/mL</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>12 mg/dL</td>
<td>Valproic Acid</td>
<td>50 mg/dL</td>
</tr>
<tr>
<td>Heparin¹ (Low MW)</td>
<td>3000 units/L</td>
<td>Mycophenolic Acid</td>
<td>1800 µg/mL</td>
</tr>
</tbody>
</table>

¹ Low molecular weight (MW) range, 4000 – 6000 Da.

<table>
<thead>
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<th>Test Compound</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Heparin¹ (High MW)</td>
<td>3000 units/L</td>
<td>Mycophenolic Acid</td>
<td>1800 µg/mL</td>
</tr>
<tr>
<td>Kanamycin B Sulfate</td>
<td>6 mg/dL</td>
<td>Glucuronide</td>
<td></td>
</tr>
<tr>
<td>Lidocaine</td>
<td>6 mg/dL</td>
<td>Phenobarbital</td>
<td>15 mg/dL</td>
</tr>
<tr>
<td>Mycophenolic Acid</td>
<td>500 µg/mL</td>
<td>Spectinomycin</td>
<td>100 µg/mL</td>
</tr>
</tbody>
</table>

¹ High MW range, 17000 – 19000 Da.

### POTENTIALLY INTERFERING ENDOGENOUS SUBSTANCES

Whole blood specimens spiked with cyclosporine targeting concentrations between 70 and 900 ng/mL were supplemented with the following potentially interfering endogenous substances. The mean recoveries for the following substances tested ranged from 92% to 110%.*

<table>
<thead>
<tr>
<th>Potentially Interfering Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>25%, 55%</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>40 mg/dL</td>
</tr>
<tr>
<td>Total Protein</td>
<td>12 g/dL</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>20 mg/dL</td>
</tr>
</tbody>
</table>

Observed mean recoveries for the following substances tested during the study ranged from 101% to 117%.*

<table>
<thead>
<tr>
<th>Potentially Interfering Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>1500 mg/dL</td>
</tr>
<tr>
<td>Total Protein</td>
<td>3 g/dL</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>500 mg/dL</td>
</tr>
</tbody>
</table>

### POTENTIALLY INTERFERING CLINICAL CONDITIONS
The ARCHITECT Cyclosporine assay was evaluated using specimens with HAMA and rheumatoid factor (RF) to further assess the clinical specificity. Five specimens positive for HAMA and five specimens positive for RF were evaluated for % recovery with cyclosporine spiked into each specimen targeting 70 and 900 ng/mL. Mean percent recovery results are summarized in the following table.*

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>Number of Specimens</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAMA</td>
<td>5</td>
<td>98</td>
</tr>
<tr>
<td>RF</td>
<td>5</td>
<td>97</td>
</tr>
</tbody>
</table>

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

XIV. SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Cyclosporine assay is designed to have precision of \( \leq 15\% \) total CV.

A study was performed with the ARCHITECT Cyclosporine assay based on guidance from the Clinical Laboratory and Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards [NCCLS]) document EP5-A2.23 Three levels of lyophilized multiconstituent controls 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. Data from this study are summarized in the following table.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Instrument</th>
<th>Reagent Lot</th>
<th>n</th>
<th>Mean (ng/mL)</th>
<th>Within Run SD</th>
<th>%CV</th>
<th>Total SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>92.6</td>
<td>6.8</td>
<td>7.3</td>
<td>11.3</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>92.1</td>
<td>6.9</td>
<td>7.5</td>
<td>8.0</td>
<td>8.7</td>
</tr>
<tr>
<td>Level 2</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>463.9</td>
<td>33.4</td>
<td>7.2</td>
<td>44.5</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>407.5</td>
<td>24.5</td>
<td>6.0</td>
<td>25.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Level 3</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>975.4</td>
<td>69.3</td>
<td>7.1</td>
<td>81.0</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>925.7</td>
<td>82.4</td>
<td>8.9</td>
<td>82.4</td>
<td>8.9</td>
</tr>
<tr>
<td>Panel 1</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>161.2</td>
<td>14.3</td>
<td>8.9</td>
<td>16.9</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>153.4</td>
<td>9.7</td>
<td>6.3</td>
<td>12.1</td>
<td>7.9</td>
</tr>
<tr>
<td>Panel 2</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>686.0</td>
<td>48.7</td>
<td>7.1</td>
<td>59.7</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>607.0</td>
<td>32.8</td>
<td>5.4</td>
<td>36.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Panel 3</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>88.6</td>
<td>10.7</td>
<td>12.1</td>
<td>11.3</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>87.1</td>
<td>7.6</td>
<td>8.7</td>
<td>9.9</td>
<td>11.4</td>
</tr>
<tr>
<td>Panel 4</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>261.4</td>
<td>23.8</td>
<td>9.1</td>
<td>26.1</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>231.7</td>
<td>12.5</td>
<td>5.4</td>
<td>16.9</td>
<td>7.3</td>
</tr>
<tr>
<td>Panel 5</td>
<td>1</td>
<td>1</td>
<td>80</td>
<td>949.7</td>
<td>69.3</td>
<td>7.3</td>
<td>73.1</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>80</td>
<td>877.0</td>
<td>56.1</td>
<td>6.4</td>
<td>62.3</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Specificity

A study was performed with the ARCHITECT Cyclosporine assay based on guidance from the CLSI document EP7-A2.25 Aliquots of whole blood were supplemented with cyclosporine, targeting values ranging from 30 to 1500 ng/mL. These specimens were spiked with cross-reactant solutions at the concentrations listed and tested for cyclosporine. Data from this study are summarized in the following table.*

Cyclosporine metabolites that have been detected in human blood were tested in the ARCHITECT Cyclosporine assay. Purified cyclosporine metabolites are not commercially available for cross-reactivity testing. Cyclosporine metabolite AM1 was synthesized chemically from cyclosporine powder and was analyzed by HPLC and mass spectrometry. Cyclosporine metabolites AM9 and AM4N were synthesized chemically from cyclosporine powder and were analyzed by HPLC, mass spectrometry and NMR spectroscopy. Cyclosporine metabolites AM19 and AM1c were isolated by semi-preparative HPLC from human bile from liver-grafted patients receiving cyclosporine treatment. They were analyzed by FAB mass spectrometry and NMR spectroscopy.
<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Metabolite Added (ng/mL)</th>
<th>Ranges of Mean Excess Concentration Detected (ng/mL, n = 5)</th>
<th>Ranges of % Cross Reactivitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM1</td>
<td>(M17) 1000</td>
<td>-6.8 to 16.5</td>
<td>-0.7 to 1.7</td>
</tr>
<tr>
<td>AM1c</td>
<td>(M18) 1000</td>
<td>-7.8 to 32.9</td>
<td>-0.8 to 3.3</td>
</tr>
<tr>
<td>AM4N</td>
<td>(M21) 1000</td>
<td>-23.1 to 30.6</td>
<td>-2.3 to 3.1</td>
</tr>
<tr>
<td>AM9</td>
<td>(M1) 1000</td>
<td>-37.6 to 19.0</td>
<td>-3.8 to 1.9</td>
</tr>
<tr>
<td>AM19</td>
<td>(M8) 1000</td>
<td>-28.9 to 21.1</td>
<td>-2.9 to 2.1</td>
</tr>
</tbody>
</table>

**Sensitivity**

The ARCHITECT Cyclosporine assay is designed to have a limit of detection (LoD) of ≤ 25.0 ng/mL, which is below the reportable range of the assay. The LoD of the ARCHITECT Cyclosporine assay, defined as the concentration at two standard deviations above the ARCHITECT Cyclosporine Calibrator A (0 ng/mL), was calculated to be 4.7 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).

**XV. ALTERNATE METHODS**

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

**XVI. REFERENCES**

ABBOTT ARCHITECT Cyclosporine package insert
Abbott Laboratories
Diagnostics Division
Abbott Park, IL 60064
PN 301-512 8/08
Revision 09/08