Cyclosporine

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

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.

Key to symbols used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>REF</td>
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<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
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<tr>
<td>Manufacturer</td>
<td></td>
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<td>Serial Number</td>
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<td>Reagent Lot</td>
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<tr>
<td>CONTROL NO.</td>
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<td>Reaction Vessels</td>
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<td>SEPTUM</td>
<td>Septum</td>
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<tr>
<td>REPLACEMENT CAPS</td>
<td>Replacement Caps</td>
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<td>Centrifuge Tubes</td>
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<td>Centrifuge</td>
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<tr>
<td>PRECISION DISPENSER</td>
<td>Precision Dispenser</td>
</tr>
<tr>
<td>STERILE</td>
<td>Sterile, method of sterilization using irradiation</td>
</tr>
<tr>
<td>R</td>
<td>Do not re-use</td>
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</table>

See REAGENTS section for a full explanation of symbols used in reagent component naming.
NAME
ARCHITECT Cyclosporine

INTENDED USE
The ARCHITECT Cyclosporine assay is a chemiluminescent microplate immunoassay (CMA) 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. The use of cyclosporine is associated with serious toxic side effects, primarily nephrotoxicity and hepatotoxicity. Many drugs affect cyclosporine blood concentrations. These drugs alter cyclosporine blood concentrations by inducing drug metabolism, interfering with drug metabolism, or affecting drug absorption. The ARCHITECT Cyclosporine assay is used as an aid in the management of heart, liver and kidney transplant patients receiving cyclosporine therapy.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE
The ARCHITECT Cyclosporine assay is a two-step immunoassay for the quantitative determination of cyclosporine in human whole blood using CMA 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 lysed with a solubilization reagent, extracted with a precipitation reagent and centrifuged. The supernatant is decanted into a Transplant Pretreatment Tube, which is placed onto the ARCHITECT i System. The microparticles, conjugate and assay diluent contain cyclosporine present in the sample binds to the anti-cyclosporine coated microparticles. After washing, cyclosporine acridinium-labeled conjugate is added to create a reaction mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). An indirect relationship exists between the amount of cyclosporine in the sample and the RLUs detected by the ARCHITECT / System optics. For additional information on system and assay technology refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS
Reagent Kit, 100 Tests
Note: Some kit sizes are not available in all countries or for use on all ARCHITECT i Systems. Please contact your local distributor.
ARCHITECT Cyclosporine Reagent Kit (1L75)
- **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.
- **CONJUGATE** 1 Bottle (12.0 mL) Cyclosporine acridinium-labeled conjugate in citrate buffer with detergent. Preservative: ProClin 300.
- **ASSAY DILUENT** 1 Bottle (10.0 mL) Assay Diluent containing MES buffer and NaCl. Preservative: ProClin 300.
- **PRE-TRIGGER SOLUTION** Pre-trigger solution containing 1.32% (w/v) hydrogen peroxide.
- **TRIGGER SOLUTION** Trigger solution containing 0.35 N sodium hydroxide.
- **WASH BUFFER** Wash buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS
- **IVD**
- **For In Vitro Diagnostic Use**
- 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.

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, Bio-Safety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- 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.

- For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.
- For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions
- Do not use reagent kits beyond the expiration date.
- Do not pool reagents within a kit or between reagent kits.
- Before 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.
- 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 the reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

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

For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

The default result unit for the ARCHITECT Cyclosporine assay is "µg/L". When the alternate result unit "mg/L" is selected, the conversion factor used by the system is 0.831525. Conversion Formula: (Concentration in ng/mL) x 1.0 = µg/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

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

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

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

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.

Specimen Conditions
Do not use specimens with the following conditions:
- heat-inactivated specimens
- obvious microbial contamination
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis
- Follow the tube manufacturer’s processing instructions for whole blood collection tubes.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- For the Manual Pretreatment Procedure in the PROCEDURE section.

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

Avoid multiple freeze/thaw cycles.

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.

Shipping
When shipping specimens, package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

Specimens may be shipped on wet ice or on dry ice. Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided
- 1L75 ARCHITECT Cyclosporine Reagent Kit
- 1L75-55 ARCHITECT Cyclosporine Whole Blood Precipitation Reagent Kit
- 9527-40 XSYSTEMS CENTRIFUGE TUBES
- 1P06-01 Transplant Pretreatment Tubes

Materials Required but not Provided
- ARCHITECT i System
- 3K50 ARCHITECT i ASSAY CD-ROM - US - Addition A
- 3K52 ARCHITECT i ASSAY CD-ROM - WW (excluding US) - Addition A
- 1L75-01 ARCHITECT Cyclosporine Calibrators
- 1P05-10 Abbott Immunosuppressant-MCC or other commercial controls
- Vortex Mixer
- 9527-26 XSYSTEMS PRE-TRIGGER SOLUTION
- ARCHITECT TRIGGER SOLUTION
- ARCHITECT WASH BUFFER
- ARCHITECT REACTION VESSELS
- ARCHITECT SEPULT
- ARCHITECT REPLACEMENT CAPS
- Precision Micropipettes
- Pipette tips
- 9528-02 XSYSTEMS PRECISION DISPENSER, or equivalent
- 2.5 mL Combips, or equivalent, for dosing with the MM Dispenser

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

Manual Pretreatment Procedure
The ARCHITECT Cyclosporine assay requires a manual pretreatment step for all whole blood patient specimens, ARCHITECT Cyclosporine Calibrators and Abbott Immunosuppressant-MCC or other controls.

Use only ARCHITECT Cyclosporine Whole Blood Precipitation Reagent Kit (1L75-55).

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

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

Manual Pretreatment Procedure

Attention: To obtain optimal results for the ARCHITECT Cyclosporine 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 200 µL of each sample into an XSYSTEMS Centrifuge Tube immediately after mixing. Use a different 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. 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 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.

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 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.

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 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. Only an even number of tubes can be centrifuged at one time.

Centrifuge the tubes for 4 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 / System is ready to accept samples.

Warning: Do not disturb the pellet. Do not pipette the supernatant as this will help ensure 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 / 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.

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.

Assay Procedure

• 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. After the first time the microparticles have been loaded, no further mixing is required.
  • Invert the microparticle bottle 30 times.
  • Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue inverting the bottle until the microparticles have been completely resuspended.
  • If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.
  • Once the microparticles have been resuspended, place a septum on the bottle. For instructions on placing septums on bottles refer to the Handling Precautions section of this package insert.
• Load the ARCHITECT Cyclosporine Reagent Kit on the ARCHITECT / System.
  • Verify that all necessary assay reagents are present.
  • Ensure that septums are present on all reagent bottles.
• Order calibration, if necessary.
  • For information on ordering 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 four replicates may be sampled from the same Transplant Pretreatment Tube.
• 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 / System.
• With the Transplant Pretreatment Tube, use the sample gauge to ensure sufficient patient specimen is present for the ARCHITECT Cyclosporine 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.
The measurement range of the ARCHITECT Cyclosporine assay is 0 - 1500 ng/mL.

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Section 6.

Calibration

- 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.
- Calibration Range: 0 - 1500 ng/mL.
- Once an ARCHITECT Cyclosporine calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT Cyclosporine assay is that a single sample of each control level be tested once every 24 hours each day of use. Commercial controls such as the Abbott Immunosuppressant-MCC are suitable for this purpose. If the quality control procedures in your laboratory require more frequent use of controls to verify assay results, follow those procedures. Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated test results are invalid and 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 Cyclosporine assay belongs to method group 6. ARCHITECT Cyclosporine Calibrators may be used when MasterCheck is not available. Refer to the ARCHITECT System Operations Manual, Appendix B.

RESULTS

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

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measurement Range (Reportable Range)

The measurement range of the ARCHITECT Cyclosporine assay is 30.0 ng/mL (minimum reportable value based on Functional Sensitivity) to 1500.0 ng/mL.

LIMITATIONS OF THE PROCEDURE

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- If the cyclosporine results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- 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.
- Immunassays 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.
- 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.
- 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.

EXPECTED VALUES

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 regimen 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.

SPECIFIC PERFORMANCE CHARACTERISTICS

Performance was evaluated on the ARCHITECT i2000 and i2000SR Systems.

Precision

The ARCHITECT Cyclosporine assay is designed to have precision of ≤ 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 EPS-A2. Three levels of lyophilized multiclonal 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>
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<th>Level</th>
<th>Sample</th>
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<th>2</th>
<th>3</th>
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<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
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<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>80</td>
<td>92.1</td>
<td>6.9</td>
<td>75</td>
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<tr>
<td>2</td>
<td>1</td>
<td>80</td>
<td>463.9</td>
<td>33.4</td>
<td>42.4</td>
<td>8.9</td>
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<tr>
<td>3</td>
<td>1</td>
<td>80</td>
<td>975.4</td>
<td>69.3</td>
<td>81.0</td>
<td>8.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

   Mean (ng/mL) SD %CV SD %CV SD %CV

SPECIFICITY

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.

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

Recovery
The ARCHITECT Cyclosporine assay is designed to have a mean recovery of 100 ± 10% of the expected value. A study was performed where known concentrations of cyclosporine were added to aliquots of whole blood specimens. The concentration of cyclosporine was determined using the ARCHITECT Cyclosporine assay and the resulting percent recovery was calculated. Measuring replicate samples may improve accuracy of results.

Dilution Linearity
The ARCHITECT Cyclosporine assay is designed to have a mean recovery of 100 ± 10% of the expected value. A study was performed where known concentrations of cyclosporine were added to aliquots of whole blood specimens. The concentration of cyclosporine was determined using the ARCHITECT Cyclosporine assay and the resulting percent recovery was calculated. Measuring replicate samples may improve accuracy of results.

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).

Specificity
A study was performed with the ARCHITECT Cyclosporine assay based on guidance from the CLSI document EP7-A2. 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 liver-grafted patients receiving cyclosporine treatment. They were analyzed by FAB mass spectrometry and NMR spectroscopy.


dilution factor

\[
\text{Dilution Factor} = \frac{\text{Undiluted Observed Concentration}}{\text{Diluted Expected Concentration}}
\]

\[
\text{Expected Concentration} = \frac{\text{Undiluted Observed Concentration}}{\text{Dilution Factor}}
\]

\[
\text{% Deviation from Linearity} = \frac{\text{Observed Concentration} - \text{Expected Concentration}}{\text{Expected Concentration}} \times 100
\]

Ranges of Mean

\[
\text{Ranges of } \% \text{ Cross Reactivity} = \frac{\text{Observed Concentration} - \text{Expected Concentration}}{\text{Expected Concentration}} \times 100
\]

Ranges of Mean

\[
\text{Ranges of } \% \text{ Excess Concentration} = \frac{\text{Observed Concentration} - \text{Expected Concentration}}{\text{Expected Concentration}} \times 100
\]
**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 89% to 109%.*

<table>
<thead>
<tr>
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<th>Test Conc.</th>
<th>Test Compound</th>
<th>Test Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>20 µg/mL</td>
<td>Hydrocortisone</td>
<td>1.2 µg/mL</td>
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<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 µg/mL</td>
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<td>6 µg/mL</td>
</tr>
<tr>
<td>Amikacin*H2O</td>
<td>15 µg/mL</td>
<td>Ketoconazole</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>5.6 µg/mL</td>
<td>Labetalol</td>
<td>171 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/mL</td>
<td>Minoxidil</td>
<td>60 µg/mL</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>8 µg/mL</td>
<td>N-Acetyl-</td>
<td>12 mg/dL</td>
</tr>
<tr>
<td>Carisoprodol</td>
<td>12 mg/dL</td>
<td>Naloxone</td>
<td>1.2 µg/mL</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25 µg/mL</td>
<td>Nardilone</td>
<td>25 µg/mL</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>1.5 µg/mL</td>
<td>Phenolamine</td>
<td>10 mg/dL</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>10 µg/mL</td>
<td>Prazosin</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>7.4 µg/mL</td>
<td>Prednisolone</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.01 µg/mL</td>
<td>Prednisone</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Colchicine</td>
<td>0.09 µg/mL</td>
<td>Prinidine</td>
<td>10 mg/dL</td>
</tr>
<tr>
<td>Dicloterol</td>
<td>1.2 µg/mL</td>
<td>Prochlorperid</td>
<td>600 µg/mL</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>80 µg/mL</td>
<td>Propranolol</td>
<td>5 mg/dL</td>
</tr>
<tr>
<td>Diphenylalkane</td>
<td>4.8 µg/mL</td>
<td>Propafenone</td>
<td>200 µg/mL</td>
</tr>
<tr>
<td>Diuretics</td>
<td>60 µg/dL</td>
<td>Quinidine</td>
<td>5 mg/dL</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>3 µg/mL</td>
<td>Ranitidine</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>20 µg/mL</td>
<td>Rifampin</td>
<td>5 mg/dL</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>30 µg/mL</td>
<td>Sirolimus</td>
<td>60 mg/dL</td>
</tr>
<tr>
<td>Fluorocaine</td>
<td>40 µg/mL</td>
<td>Tacrolimus</td>
<td>0.06 µg/mL</td>
</tr>
<tr>
<td>Furosemide</td>
<td>2 µg/mL</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>5000 units/L</td>
<td>Vancomycin</td>
<td>10 µg/mL</td>
</tr>
<tr>
<td>Heparin1 (High MW)</td>
<td>3000 units/L</td>
<td>Mycophenolic Acid</td>
<td>1800 µg/mL</td>
</tr>
<tr>
<td>Heparin1 (Kanamycin B Sulfate)</td>
<td>6 mg/dL</td>
<td>Glucuronide</td>
<td>1500 µg/mL</td>
</tr>
<tr>
<td>Heparin1 (Lidocaine)</td>
<td>6 mg/dL</td>
<td>Meclofenamic Acid</td>
<td>1500 µg/mL</td>
</tr>
<tr>
<td>Heparin1 (Myophenolic Acid)</td>
<td>500 µg/mL</td>
<td>Mycophenolic Acid</td>
<td>1800 µg/mL</td>
</tr>
</tbody>
</table>

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

**Observed mean recoveries for the following compounds tested during the study ranged from 89% to 109%.***

- **Heparin1** (Low MW): 5000 units/L
- **Heparin1** (High MW): 3000 units/L
- **Heparin1** (Kanamycin B Sulfate): 6 mg/dL
- **Heparin1** (Lidocaine): 6 mg/dL
- **Heparin1** (Myophenolic Acid): 500 µg/mL

**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 82% 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>

**Method Comparison**

The ARCHITECT Cyclosporine assay is designed to have a correlation coefficient of ≥ 0.90 for specimens between 30 - 1500 ng/mL when compared to the TDx/TDxFLx Cyclosporine Monoclonal Whole Blood assay.

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

**ARCHITECT Cyclosporine vs. TDx/TDxFLx Cyclosporine Monoclonal Whole Blood**

| Number of Observations | Intercept (95% CI) | Slope (95% CI) | Correlation Coefficient | Square Root of MSE (sy|x)^b |
|------------------------|-------------------|---------------|-------------------------|---------------------------|
| 227                    | -24.65            | 0.93          | 0.99                    | 53.46                     |

Specimen Range (ARCHITECT): 31.3 ng/mL to 1457.3 ng/mL

Specimen Range (TDx/TDxFLx): 44.99 ng/mL to 1437.58 ng/mL

* Confidence Interval (CI)

**Root Mean Square Error (MSE) is an estimator of the variation of the results around the fitted curve.**

**Additional testing of the above samples was completed with LC/MS/MS where regression analysis was performed using the Passing-Bablok method. Data from this study are summarized in the following table.*

**ARCHITECT Cyclosporine vs. LC/MS/MS**

| Number of Observations | Intercept (95% CI) | Slope (95% CI) | Correlation Coefficient | Square Root of MSE (sy|x)^b |
|------------------------|-------------------|---------------|-------------------------|---------------------------|
| 227                    | -24.94            | 1.20          | 0.99                    | 70.89                     |

Specimen Range (ARCHITECT): 31.3 ng/mL to 1457.3 ng/mL

Specimen Range (LC/MS/MS): 31.3 ng/mL to 1220.0 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 Cyclosporine vs. TDx/TDxFLx Cyclosporine Monoclonal Whole Blood assay was performed on the same 227 human whole blood EDTA samples in the range of 44.99 to 1437.58 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 Cyclosporine vs. TDx/TDxFLx Cyclosporine Monoclonal Whole Blood assay in this study was -53.4 ng/mL. The 95% confidence interval of the ng/mL difference bias is -60.5 ng/mL to -46.2 ng/mL. Results of the study are summarized below.*

ARCHITECT Cyclosporine ng/mL Difference from TDx Cyclosporine

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

A bias analysis of the ARCHITECT Cyclosporine vs. LC/MS/MS assay was performed on the same 227 human whole blood EDTA samples in the range of 31.3 to 1220.0 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 Cyclosporine vs. LC/MS/MS assay in this study was 44.9 ng/mL. The 95% confidence interval of the ng/mL difference bias is 33.4 ng/mL to 56.4 ng/mL. Results of the study are summarized below.*

ARCHITECT Cyclosporine ng/mL Difference from LC/MS/MS

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

BIBLIOGRAPHY


ARCHITECT, Chemiflex and TDx/TDxFLx are trademarks of Abbott Laboratories in various jurisdictions.
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