ARCHITECT Cyclosporine Reagent Kit

REF 3R30-25

en

Cyclosporine
3R30
G91292R02
B3R300

Revised September 2019.

Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

For laboratory professional use only.

NAME

ARCHITECT Cyclosporine

INTENDED USE

The ARCHITECT Cyclosporine assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of cyclosporine in human whole blood on the ARCHITECT i System.

The ARCHITECT Cyclosporine assay is to be used as an aid in the management of heart, liver and kidney transplant patients receiving cyclosporine therapy.

SUMMARY AND EXPLANATION OF THE TEST

Cyclosporine is a cyclic undecapeptide of fungal origin and a potent immunosuppressant.[1] 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.[2] Cyclosporine therapy has greatly improved organ transplant survival of heart, liver and kidney transplants.[3, 4]

Cyclosporine may be administered by IV or orally. Absorption from the gastrointestinal tract is variable, unpredictable, and incomplete.[5] Bioavailability increases during treatment so oral doses must be gradually reduced in order to maintain a constant cyclosporine concentration in the blood.[6] Assessment of cyclosporine concentrations in blood aids in adjusting patients' dosage and avoids cyclosporine underdosage inefficacy or overdosage toxicity.[7, 8] 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.[9] Preliminary data indicate cyclosporine metabolites are less immunosuppressive and less toxic than their parent compound.[10]

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.[11]

The use of cyclosporine is associated with serious toxic side effects, primarily nephrotoxicity and hepatotoxicity.[12, 13] Other adverse effects include diarrhea, gum hyperplasia, nausea, vomiting, hirsutism, tremor, and hypertension.[14] Some studies have shown the benefits of monitoring cyclosporine concentrations, including a reduction in the incidence of biopsy proven acute rejection.[15]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is an automated two-step immunoassay for the quantitative determination of cyclosporine in human whole blood using chemiluminescent microparticle immunoassay (CMIA) technology.

Prior to the initiation of the automated ARCHITECT i System 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.

Sample, anti-cyclosporine coated paramagnetic microparticles, and assay specific diluent are combined and incubated. The cyclosporine present in the sample binds to the anti-cyclosporine coated microparticles. The mixture is washed. Cyclosporine acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is an indirect relationship between the amount of cyclosporine in the sample and the RLU detected by the system optics.

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

REAGENTS

Kit Contents

ARCHITECT Cyclosporine Reagent Kit 3R30

Volumes (mL) listed in the following table indicate the volume per bottle.

REF	3R30-25
Tests per kit	100
Number of kits per box	1
Tests per box	100
MICROPARTICLES	8.0 mL
CONJUGATE	12.0 mL
ASSAY SPECIFIC DILUENT	10.0 mL

MICROPARTICLES Anti-cyclosporine (mouse, monoclonal) coated microparticles in MOPS buffer with protein (bovine) stabilizer. Minimum concentration: 0.16% solids. Preservatives: sodium azide and ProClin 950.

CONJUGATE Cyclosporine acridinium-labeled conjugate in citrate buffer with detergent. Minimum concentration: 0.60 ng/mL. Preservative: ProClin 300.

ASSAY SPECIFIC DILUENT MES buffer and NaCl. Preservative: ProClin 300.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[16-19]

The following warnings and precautions apply to: MICROPARTICLES	
	
WARNING	Contains 4-morpholinopropanesulphonic acid*, methylisothiazolone and sodium azide.
H317	May cause an allergic skin reaction.
H316*	Causes mild skin irritation.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

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

For the most current hazard information, see the product Safety Data Sheet.

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

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

Reagent Handling

- Do not pool reagents within a kit or between kits.
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the PROCEDURE, Assay Procedure section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of reagent handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Onboard	System Temperature	14 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. 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.

Reagents may be stored on or off the ARCHITECT i System. If reagents are removed from the system, store them at 2 to 8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright.

For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when a calibration error occurs or a control value is out of the specified range. 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 prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

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

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

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

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

Default result unit	Conversion factor	Alternate result unit
ng/mL	1.0	µg/L
	0.831525	nmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen type listed below was verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Whole Blood	EDTA

- 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 concentration values for individual specimens.

The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Do not use:
  - heat-inactivated specimens
  - specimens with obvious microbial contamination
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.

For preparation instructions, follow the Manual Pretreatment Procedure in the PROCEDURE section of this package insert.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time
Whole Blood	2 to 8°C	7 days

If testing will be delayed longer than the maximum 2 to 8°C storage time, store frozen (-10°C or colder).

Avoid more than 3 freeze/thaw cycles.

Specimens must be mixed thoroughly after thawing to ensure consistency of the results.

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 of this package insert be repeated.

Specimen Shipping

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

Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

- 3R30 ARCHITECT Cyclosporine Reagent Kit

Materials Required but not Provided

- ARCHITECT Cyclosporine assay file obtained from the ARCHITECT i System e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 1L75-55 ARCHITECT Cyclosporine Whole Blood Precipitation Reagent Kit
- 1P06 Transplant Pretreatment Tubes
- 3R30-01 ARCHITECT Cyclosporine Calibrators
- Controls containing cyclosporine
- Vortex Mixer
- Laboratory microcentrifuge
- Polypropylene Centrifuge Tubes compatible with laboratory microcentrifuge
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Septum
- Precision Micropipettes
- Precision Dispenser, or equivalent
- 2.5 mL Combitips, or equivalent, for Precision Dispenser
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

For information on materials required for operation of the instrument, refer to the ARCHITECT System Operations Manual, Section 1.

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 controls containing cyclosporine.

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 Sample Dilution Procedures section of this package insert.

Warning: Only Transplant Pretreatment Tubes (LN 1P06) 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.

Note: A Cyclosporine Sample Pretreatment Guide outlining the pretreatment steps is also available from the technical library at www.corelaboratory.abbott or from 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 a microcentrifuge tube or equivalent polypropylene centrifuge tube (e.g. round bottom) immediately after mixing. Use a different tube for each sample.

Note: A new pipette tip **must** be used each time **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 a microcentrifuge taking care to balance the rotor. A balance tube can be added if necessary. Only an even number of tubes can be centrifuged at one time.

   Centrifuge the tubes for a minimum of 4 minutes at > 9500 x g RCF, or 38 500 g-minutes.
6. **Remove** each tube from the centrifuge and **inspect** for the presence of a well-formed pellet and clear supernatant.
7. **Uncap** each tube and **decant** (pour off) the supernatant into the **Transplant Pretreatment Tube** when the ARCHITECT i 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) 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.

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

For a detailed description of how to run an assay, refer to the ARCHITECT System Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the ARCHITECT System Operations Manual, Section 5 to ensure sufficient specimen is present.
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the Reagent Handling section of this package insert.
- No more than 12 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 i System.
  - With the Transplant Pretreatment Tube, use the sample gauge to ensure sufficient patient specimen is present for the ARCHITECT Cyclosporine assay.
- Refer to the ARCHITECT Cyclosporine calibrator package insert for usage. Refer to the Manual Pretreatment Procedure section of this reagent package insert for the preparation of the calibrators.
- For general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples with a cyclosporine value exceeding 1500.0 ng/mL (1247.3 nmol/L) are flagged with the code "> 1500.0 ng/mL" ("> 1247.3 nmol/L") and may be diluted with the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution: 1:2

Specimen must be diluted before pretreatment.

Add 150 µL of the sample to 150 µL of ARCHITECT Cyclosporine Calibrator A, then proceed with the Manual Pretreatment Procedure in the PROCEDURE section of this package insert.

The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result.

The result should be > 200.0 ng/mL (> 166.3 nmol/L) before the dilution factor is applied.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than 200.0 ng/mL (166.3 nmol/L), do not report the result. Rerun using an appropriate dilution.

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

Calibration

For instructions on performing a calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Each level of cyclosporine control must be tested to evaluate the assay calibration.

Once a 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.
- Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.
    - If statistically-based quality control limits are not available, then the calibration should not exceed a 30-day limit for recalibration frequency.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

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.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24, 4th ed., or other published guidelines, for general quality control recommendations.[20]

- If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.
- If quality control results do not meet the acceptance criteria defined by your laboratory, sample results may be suspect. Follow the established quality control procedures for your laboratory. Recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[21]

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.

RESULTS

Calculation

The ARCHITECT Cyclosporine assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

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.

Reportable Interval

Based on representative data for the limit of quantitation (LoQ) and the limit of detection (LoD), the ranges over which results can be reported are provided below according to the definitions from CLSI EP34, 1st ed.[22]

	ng/mL	nmol/L
Analytical Measuring Interval (AMI)[a]	18.0 - 1500.0	15.0 - 1247.3
Extended Measuring Interval (EMI)[b]	1500.0 - 3000.0	1247.3 - 2494.6
Reportable Interval[c]	6.7 - 3000.0	5.6 - 2494.6

[a] AMI: The AMI extends from the LoQ to the upper limit of quantitation (ULoQ). This is determined by the range of values in ng/mL (nmol/L) that demonstrated acceptable performance for linearity, imprecision, and bias.

[b] EMI: The EMI extends from the ULoQ to the ULoQ × dilution factor. The value reflects a 1:2 dilution factor.

[c] The reportable interval extends from the LoD to the upper limit of the EMI.

Note: The default Low Linearity value of the assay file corresponds to 6.7 ng/mL (5.6 nmol/L).

LIMITATIONS OF THE PROCEDURE

- Do not use ARCHITECT Sample Cups for whole blood samples. Refer to the Assay Procedure section of this package insert for further information.
- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the cyclosporine results are inconsistent with clinical evidence, additional testing is recommended.

- 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.
- Potential interference has not been evaluated for substances other than those described in the SPECIFIC PERFORMANCE CHARACTERISTICS, Interference section of this package insert.
- 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 SPECIFIC PERFORMANCE CHARACTERISTICS, Analytical Specificity section of this package insert 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). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT Cyclosporine that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[23, 24]
- 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.[25]

EXPECTED VALUES

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

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. When changing cyclosporine assay methods, including between Abbott assays, in the course of monitoring a patient, additional sequential testing should be carried out to confirm baseline values.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A3.[26] Testing was conducted using 3 lots of the ARCHITECT Cyclosporine Reagent Kit, 3 lots of the ARCHITECT Cyclosporine Calibrators, and 1 lot of commercially available controls and 2 instruments. Three controls and 5 human whole blood panels were tested in duplicate, twice per day on 20 days on 6 instrument/reagent/calibrator lot combinations, where a unique reagent lot and a unique calibrator lot were paired with 1 instrument. The performance from a representative combination is shown in the following tables.

			Within-Run (Repeatability)		Within-Laboratory[a]	
Sample	n	Mean (ng/mL)	SD	%CV	SD (Range[b])	%CV (Range[b])
Level 1	120	80.4	3.08	3.8	3.64 (3.64-4.16)	4.5 (4.5-5.3)
Level 2	120	316.1	11.14	3.5	16.68 (16.68-21.05)	5.3 (5.3-6.6)
Level 3	119	643.3	23.10	3.6	52.63 (49.20-57.12)	8.2 (6.9-8.8)
Panel 1	119	41.6	2.19	5.3	2.40 (2.40-3.55)	5.8 (5.8-9.0)
Panel 2	120	69.0	2.68	3.9	3.66 (3.42-3.99)	5.3 (5.0-5.8)
Panel 3	120	238.5	7.23	3.0	8.40 (8.40-11.30)	3.5 (3.5-4.5)
Panel 4	119	657.1	16.75	2.5	25.76 (25.76-33.20)	3.9 (3.9-4.6)
Panel 5	120	1357.9	59.46	4.4	77.69 (65.40-90.70)	5.7 (4.7-6.5)

[a] Includes within-run, between-run, and between-day variability.
[b] Minimum and maximum SD or %CV across all reagent lot and instrument combinations.

			Within-Run (Repeatability)		Within-Laboratory[a]	
Sample	n	Mean (nmol/L)	SD	%CV	SD (Range[b])	%CV (Range[b])
Level 1	120	66.9	2.56	3.8	3.03 (3.02-3.46)	4.5 (4.5-5.3)
Level 2	120	262.8	9.27	3.5	13.87 (13.87-17.50)	5.3 (5.3-6.6)
Level 3	119	534.9	19.20	3.6	43.76 (40.92-47.50)	8.2 (6.9-8.8)
Panel 1	119	34.6	1.82	5.3	2.00 (2.00-2.95)	5.8 (5.8-9.0)
Panel 2	120	57.4	2.22	3.9	3.04 (2.85-3.31)	5.3 (5.0-5.8)
Panel 3	120	198.3	6.01	3.0	6.99 (6.99-9.40)	3.5 (3.5-4.5)
Panel 4	119	546.4	13.93	2.5	21.42 (21.42-27.61)	3.9 (3.9-4.6)
Panel 5	120	1129.1	49.44	4.4	64.61 (54.38-75.42)	5.7 (4.7-6.5)

[a] Includes within-run, between-run, and between-day variability.
[b] Minimum and maximum SD or %CV across all reagent lot and instrument combinations.

Accuracy by Recovery

Testing was conducted using 1 lot of the ARCHITECT Cyclosporine Reagent Kit, 1 lot of the ARCHITECT Cyclosporine Calibrators, 1 lot of the commercially available controls, and 1 instrument. Thirty-nine unique human whole blood EDTA samples were prepared by spiking known concentrations of cyclosporine into analyte-free human whole blood within the measuring interval. The concentration of cyclosporine was determined using the ARCHITECT Cyclosporine assay and the summary of percent recovery for each sample set was calculated.

Sample Set	n	Added Cyclosporine Concentration Range (ng/mL)	Mean Test Concentration Range (ng/mL)	% Recovery[a] Range
Low	13	54.00 – 69.00	50.40 – 71.32	93 - 109
Medium	13	105.00 – 340.00	114.22 – 327.52	95 – 109
High	13	360.00 – 1400.00	350.44 – 1372.54	90 - 100

[a] $\% \text{ Recovery} = \frac{\text{Mean Test Concentration}}{\text{Added Cyclosporine Concentration}} \times 100$

Sixteen human whole blood EDTA samples from patients taking cyclosporine with initial analyte concentrations ranging from approximately 50.0 ng/mL to 800.0 ng/mL were supplemented with an additional 200.0 ng/mL of cyclosporine. The test and reference (non-spiked) samples were tested and the observed recovery values ranged from 57.2% to 97.1%.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.[27] Testing was conducted using 3 lots of the ARCHITECT Cyclosporine Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) values are summarized below.

	ng/mL	nmol/L
LoB[a]	3.3	2.7
LoD[b]	6.7	5.6
LoQ[c]	18.0	15.0

[a] The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b] The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ presented in the table is in alignment with the low end of the AMI for the ARCHITECT Cyclosporine assay on the ARCHITECT i System. The observed LoQ on the ARCHITECT i System was 11.9 ng/mL (9.9 nmol/L). This LoQ is defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met and was determined from n ≥ 60 replicates of low-analyte level samples.

Linearity

A study was performed based on guidance from CLSI EP06-A.[28] This assay is linear across the analytical measuring interval of 18.0 to 1500.0 ng/mL (15.0 to 1247.3 nmol/L).

Analytical Specificity

Interference

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07, 3rd ed.[29] Each substance was tested at 2 levels of the analyte (approximately 70 ng/mL and 800 ng/mL). No significant interference (interference within ± 10% [based on 95% Confidence Intervals]) was observed at the following concentrations.

Potentially Interfering Substance	Interferent Level
Albumin	12 g/dL
Bilirubin, Conjugated	60 mg/dL
Bilirubin, Unconjugated	40 mg/dL
Cholesterol	500 mg/dL
Gamma Globulin (at 70 ng/mL of cyclosporine)	9 g/dL
Gamma Globulin (at 800 ng/mL of cyclosporine)	10 g/dL
Hematocrit	15%
Hematocrit	60%
Triglycerides	1500 mg/dL
Uric Acid	20 mg/dL

Interference beyond ± 10% (based on 95% Confidence Intervals [CI]) was observed at the concentrations and analyte levels shown below for the following substance.

Potentially Interfering Substance	Interferent Level	Analyte Level	% Interference (95% CI)
Gamma Globulin	10 g/dL	70 ng/mL	-7.9% (-10.8, -5.0)
Gamma Globulin	12 g/dL	800 ng/mL	-12.8% (-14.9, -10.7)

Potentially Interfering Other Conditions

Fifteen specimens containing rheumatoid factor (RF) and 14 specimens containing human anti-mouse antibodies (HAMA) were evaluated in the study. Each substance was tested at 2 levels of the analyte. A mean % recovery of 100% ± 10% was observed at the following concentrations.

Clinical Condition	n	Interferent Concentration Range	Added Analyte Level	Mean % Recovery	% Recovery Range
RF	15	138.9 - 604.0 IU/mL	41.0 ng/mL	100.2	91.4-112.7
			643.5 ng/mL	104.2	97.9-110.3
HAMA	14	47.3 - 448.9 ng/mL	59.6 ng/mL	97.3	73.3-124.7
			899.0 ng/mL	102.2	90.7-120.7

Potentially Interfering Drugs

A study was performed based on guidance from CLSI EP07, 3rd ed.[29] Each substance was tested at 2 levels of the analyte (approximately 70 ng/mL and 800 ng/mL). No significant interference (interference within ± 10% [based on 95% CI]) was observed at the following concentrations.

Potentially Interfering Substance	Interferent Level
Acetaminophen	20 mg/dL
Acyclovir	3.3 µg/mL
Allopurinol	5 mg/dL
Amikacin	15 mg/dL
Amphotericin B	6.0 µg/mL
Apresoline	100 µg/mL
Azathioprine	1 mg/dL
Biotin	4300 ng/mL
Bromocriptine	8 µg/mL
Carbamazepine	12 mg/dL
Cephalosporine	101 µg/mL
Chloramphenicol	25 mg/dL
Chloroquine	1.6 µg/mL
Cimetidine	10 mg/dL
Ciprofloxacin	7.6 µg/mL
Clonidine	0.01 µg/mL
Cortisone	1.3 µg/mL
Digitoxin	85 ng/mL
Digoxin	5.0 ng/mL
Diltiazem	62 µg/mL
Dipyridamole	100 µg/mL
Disopyramide	3 mg/dL
K_2 EDTA	9 mg/mL
K_3 EDTA	9 mg/mL
Erythromycin	20 mg/dL
Ethosuximide	101 µg/mL
Everolimus	820 ng/mL
Fluconazole	30 µg/mL
Flucytosine	40 µg/mL
Furosemide	2 mg/dL
Ganciclovir	1005 µg/mL
Gemfibrozil	102 µg/mL
Gentamicin	12 mg/dL
Heparin (Low Molecular Weight)	3035 units/L
Heparin (High Molecular Weight)	3060 units/L
Hydrocortisone	1.3 µg/mL
Itraconazole	20 µg/mL
Kanamycin Sulfate	6 mg/dL
Ketoconazole	50 µg/mL
Labetalol	17.6 µg/mL
Lidocaine	6 mg/dL
Lincomycin	100 µg/mL
Lovastatin	20 µg/mL
Methotrexate	100 µg/mL
Methylprednisolone	100 µg/mL

Potentially Interfering Substance	Interferent Level
Mycophenolic Acid	503 µg/mL
Mycophenolic Acid Glucuronide	1928 µg/mL
N-Acetyl-Procainamide	12 mg/dL
Nadolol	1.3 µg/mL
Nicardipine	0.6 µg/mL
Penicillin G Na+	100 µg/mL
Phenobarbital	15 mg/dL
Phenytoin	10 mg/dL
Pimecrolimus	6 ng/mL
Prazosin	26 µg/mL
Prednisolone	101 µg/mL
Prednisone	101 µg/mL
Primidone	10 mg/dL
Procainamide	10 mg/dL
Propranolol	0.5 mg/dL
Quinidine	5 mg/dL
Rifampin	5 mg/dL
Salicylic Acid	504 µg/mL
Sirolimus (Rapamycin)	62 ng/mL
Spectinomycin	101 µg/mL
Tacrolimus	0.06 µg/mL
Theophylline	251 µg/mL
Tobramycin	2 mg/dL
Triamterene	100 µg/mL
Trimethoprim	41 µg/mL
Valproic Acid	52 mg/dL
Vancomycin	6 mg/dL
Verapamil	10 µg/mL

Other Specimen Conditions or Disease States

The ARCHITECT Cyclosporine assay was evaluated for potential interference using specimens obtained from individuals with unrelated medical conditions. Each sample was tested at 2 levels of the analyte (approximately 70 ng/mL and 800 ng/mL) to determine the percentage of cyclosporine recovered after spiking.

		Mean % Recovery		% Recovery Range	
Category	n	70 ng/mL	800 ng/mL	70 ng/mL	800 ng/mL
Anti-dsDNA autoantibodies	12	99.0	101.7	83.7 - 105.5	89.9 – 110.4
Flu vaccine recipients	12	102.9	101.6	90.7 - 110.5	92.6 – 114.1
Multiparous females ≥ 2 full term pregnancies	12	104.9	104.5	96.4 - 113.8	90.7 – 118.0
Renal disease patients	12	101.8	102.8	88.1 - 111.5	82.6 – 112.5

Cross-Reactants

A study was performed based on guidance from CLSI EP07, 3rd edition.[29] Each cross-reactant was tested at 200.0 ng/mL of cyclosporine. The results are shown below.

Cross-Reactant	Cross-Reactant Level	% Cross-Reactivity (95% CI)
AM1	1000 ng/mL	-0.1 (-0.5, 0.4)
AM9	1000 ng/mL	5.6 (4.7, 6.4)
AM19	1000 ng/mL	-0.2 (-0.7, 0.2)
AM1C9	1000 ng/mL	-6.5 (-7.0, -5.9)
AM4N	1000 ng/mL	-2.5 (-2.8, -2.1)

Method Comparison

A study was performed based on guidance from CLSI EP09-A3[30] using the Weighted Deming regression method. The study was performed to compare the ARCHITECT Cyclosporine assay to the ARCHITECT Cyclosporine assay (List Number 1L75) using human whole blood specimens from heart, kidney, and liver transplant patients receiving cyclosporine therapy.

ARCHITECT Cyclosporine vs ARCHITECT Cyclosporine (List Number 1L75)						
	Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Heart	ng/mL (nmol/L)	52	0.99	8.95 (7.44)	0.88	23.0 - 1317.3 (19.1 - 1095.4)
Kidney	ng/mL (nmol/L)	55	0.99	9.66 (8.04)	0.88	24.8 – 1174.1 (20.6 – 976.3)
Liver	ng/mL (nmol/L)	55	0.99	8.93 (7.42)	0.88	52.7 - 1140.1 (43.8 – 948.1)
Overall	ng/mL (nmol/L)	162	0.99	9.11 (7.57)	0.88	23.0 - 1317.3 (19.1 - 1095.4)

A study was performed based on guidance from CLSI EP21-A[31] for the estimation of total analytical error and CLSI EP09-A3[30] using the Weighted Deming regression method. The study was performed to compare the ARCHITECT Cyclosporine assay to LC/MS/MS using human whole blood specimens from heart, kidney, and liver transplant patients receiving cyclosporine therapy. In this study, the total error interval containing at least 95% of the distribution of the % differences for all transplant specimens across the analytical measuring interval was determined to be -30.0% to 29.0%.

ARCHITECT Cyclosporine vs LC/MS/MS						
	Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Heart	ng/mL (nmol/L)	48	0.99	10.34 (8.60)	0.88	19.0 – 1231.0 (15.8 – 1023.6)
Kidney	ng/mL (nmol/L)	55	0.98	9.36 (7.79)	0.84	28.0 – 1293.5 (23.3 – 1075.6)
Liver	ng/mL (nmol/L)	55	0.98	18.30 (15.21)	0.81	42.5 – 1236.0 (35.3 – 1027.8)
Overall	ng/mL (nmol/L)	158	0.98	11.55 (9.60)	0.84	19.0 – 1293.5 (15.8 – 1075.6)

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Note for number formatting:

- A space is used as thousands separator (example: 10 000 specimens).
- A period is used to separate the integer part from the fractional part of a number written in decimal form (example: 3.12%).

Key to Symbols

ISO 15223 Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
REF	List Number
SN	Serial number

Other Symbols

ASSAY SPECIFIC DILUENT	Assay Specific Diluent
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
MICROPARTICLES	Microparticles
PRODUCT OF IRELAND	Product of Ireland
REAGENT LOT	Reagent Lot
Rx ONLY	For use by or on the order of a physician only (applicable to USA classification only).


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