Procedure #4840-CH-501

 Architect Vancomycin



**i-Vancomycin**

**Serum/Plasma**

**Abbott Architect**

# Intended Use

The ARCHITECT iVancomycin assay is an *in vitro* chemiluminescent microparticle immunoassay (CMIA) for the quantitative measurement of vancomycin in human serum or plasma on the ARCHITECT iSystem with STAT protocol capability. The ARCHITECT iVancomycin assay is used in the diagnosis and treatment of vancomycin overdose and in monitoring levels of vancomycin to help ensure appropriate therapy.

**Clinical Significance**

Vancomycin hydrochloride is a tricyclic glycopeptide derived from *Amycolatopsis orientalis*.[*1*](#unique_6_Connect_42_li_tv3_rft_dp) It is commonly used in the treatment of methicillin-resistant *Staphylococcus aureus* infections.[*2*](#unique_6_Connect_42_li_e2l_wft_dp) This glycopeptide inhibits the growth of the bacterium by intervening in the cell wall synthesis, thereby killing the bacterium. Extensive review articles have been published which fully examine vancomycin’s effectiveness and pharmacokinetics.[*1*](#unique_6_Connect_42_li_tv3_rft_dp)*,* [*3*](#unique_6_Connect_42_li_ewy_xft_dp)

Vancomycin is absorbed minimally from the gastrointestinal tract. In the first 24 hours after intravenous dosing, the usual route of administration, about 90% of the vancomycin is excreted unchanged by the kidneys. The average half-life in patients with normal renal function is about 6 hours. Vancomycin is approximately 55% bound to plasma proteins. Therapeutic serum levels vary depending on the microorganism involved and the patient’s tolerance to the drug.[*4*](#unique_6_Connect_42_li_jy4_yft_dp)*,* [*5*](#unique_6_Connect_42_li_e55_yft_dp) Vancomycin serum or plasma concentrations are monitored to guide therapy, since individual patient differences require dose changes that are difficult to predict. Monitoring serum or plasma levels of vancomycin decreases the frequency of serious toxic effects.

# Principle

The ARCHITECT iVancomycin assay is a one-step STAT immunoassay for the quantitative measurement of vancomycin in human serum or plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample, anti-vancomycin coated paramagnetic microparticles, and vancomycin acridinium-labeled conjugate are combined to create a reaction mixture. The anti-vancomycin coated microparticles bind to vancomycin present in the sample and to the vancomycin acridinium-labeled conjugate.
2. After washing, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
3. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an indirect relationship between the amount of vancomycin in the sample and the RLUs detected by the ARCHITECT iSystem optics.

# REAGENTS

## Kit Contents

|  | 1P30-28 |
| --- | --- |
|  | 100 |
|  | 1 x 6.6 mL |
|  | 1 x 5.9 mL |
|  Anti-vancomycin (mouse, monoclonal) coated goat anti-mouse (GAM) microparticles in TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.05% solids. Preservative: ProClin 300. |
|  Vancomycin acridinium-labeled conjugate in MES buffer with surfactant. Minimum concentration: 50 ng/mL. Preservative: ProClin 300. |

## Other Reagents

|  |
| --- |
|  1 x 100 mL ARCHITECT Multi-Assay Manual Diluent,  7D82-50, containing phosphate buffered saline solution. Preservative: antimicrobial agent. |
|  ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide. |
|  ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide. |
|  ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents. |

## Warnings and Precautions

* 
* For *In Vitro* Diagnostic Use

### Safety Precautions

|  |
| --- |
| The following warnings and precautions apply to:  /  |
|  |
| **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. |

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 use reagent kits beyond the expiration date.
* **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 handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

## Reagent Storage

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

|  | Storage Temperature | Maximum Storage Time | Additional Storage Instructions |
| --- | --- | --- | --- |
| **Unopened/Opened\*** | 2-8°C | Until expiration date | May be used immediately after removal from 2-8°C storage.Store in upright position. |
| **On board** | System temperature | 30 days | Discard after 30 days. |

\* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8°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. **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.

# INSTRUMENT PROCEDURE

The ARCHITECT iVancomycin assay file must be installed on the ARCHITECT iSystem with STAT protocol capability from an ARCHITECT iSystem Assay CD-ROM prior to performing the assay.

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

# SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

## Specimen Types

Verified specimen types to be used with this assay:

| Specimen Types | Collection Tubes |
| --- | --- |
| Human serum | Serum |
| Human plasma | Lithium heparin |

* Other specimen collection tubes, including gel separation tubes, have not been tested with this assay.
* Plasma samples from different anticoagulant tube types should not be used interchangeably for monitoring vancomycin. Use of citrate should be performed only when the blood is collected in a full tube so as not to incur a dilution effect.
* Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum or plasma.
* Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens.
* The instrument does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

## Specimen Conditions

* Do not use specimens with the following conditions:
* heat-inactivated
* grossly hemolyzed
* obvious microbial contamination
* For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
* 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. Gravity separation is not sufficient for specimen preparation.
* Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
* To ensure consistency in results, centrifuge specimens before testing if
* they contain fibrin, red blood cells, or other particulate matter or
* they were frozen and thawed.
* Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
* Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

## Specimen Storage

| Specimen Type | Storage Temperature | Maximum Storage Time  |
| --- | --- | --- |
| Serum/Plasma | Room temperature | ≤ 3 days |
| 2-8°C | ≤ 8 days |
| -20°C or colder | ≤ 3 months |

Specimens may be stored on or off the clot or red blood cells for up to three days at room temperature.

Specimens removed from the clot or red blood cells may be stored up to eight days at 2-8°C.

## 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

1P30 ARCHITECT iVancomycin Reagent Kit

## Materials Required but not Provided

* ARCHITECT iVancomycin Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
* 1P30-01 ARCHITECT iVancomycin Calibrators
* Quality control products (Bio-Rad Immunoassay plus levels 1, 2 &3)
* 7D82-50 ARCHITECT Multi-Assay Manual Diluent
* ARCHITECT Pre-Trigger Solution
* ARCHITECT Trigger Solution
* ARCHITECT Wash Buffer
* ARCHITECT Reaction Vessels
* ARCHITECT Sample Cups
* ARCHITECT Septum
* ARCHITECT Replacement Caps
* Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

## Assay Procedure

* 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.
* Load the reagent kit on the ARCHITECT iSystem.
* Verify that all necessary 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.
* Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

* Priority:

Sample volume for first test: 70 μL

Sample volume for each additional test from same sample cup: 20 μL

* ≤ 3 hours on board:

Sample volume for first test: 150 μL

Sample volume for each additional test from same sample cup: 20 μL

* If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient
 specimen is present.
* Prepare ARCHITECT iVancomycin Calibrators and controls.
* Mix calibrator(s) by gentle inversion before use.
* Hold bottles **vertically** and dispense recommended volumes into each respective sample
 cup.
* Recommended volumes:

for each calibrator: 5 drops

for each control: 150 μL

* Follow the manufacturer’s instructions for preparation of commercially available control
 material.
* Load 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 System Operations Manual, Section 3.
* 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.

## Specimen Dilution Procedures

Specimens with a vancomycin value exceeding 50.00 μg/mL are flagged with the code “>50.00” and may be diluted with the Manual Dilution Procedure.

**Manual Dilution Procedure**

Suggested dilution: 1:2.

1. Add 100.00 μL of the patient specimen to 100.00 μL of ARCHITECT iVancomycin Calibrator A or ARCHITECT Multi-Assay Manual Diluent.
2. 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 > 3.0 μg/mL before the dilution factor is applied.

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

## Calibration

* Test calibrators A, B, C, D, E, and F in duplicate. The calibrators should be priority loaded.

A single sample of each vancomycin control level must be tested to evaluate the assay calibration. Ensure that assay control values are within established ranges.

* Calibration Range: 0.0 - 100.0 μg/mL.
* Once an ARCHITECT iVancomycin 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 or
* 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 iVancomycin assay is that a single sample of each control level be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific 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 sample results are invalid and the samples 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 iVancomycin assay belongs to method group 1.

# RESULTS

**Serum/Plasma:**

 **Peak:** 20 – 40 μg/mL

 **Trough:** 10 – 20 μg/mL (range established by Pharmacy)

**Critical Values:**

**Peak: >50** μg/mL

**Trough: >20** μg/mL

## Calculation

The ARCHITECT iVancomycin assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) 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.

## Measuring Interval

For the verification studies described in this package insert, **the measuring range is 3.0 μg/mL to 50.0 μg/mL.**

Measuring interval is defined as the range of values in μg/mL which meets the limits of acceptable performance for both imprecision and bias for an undiluted sample.

# LIMITATIONS OF THE PROCEDURE

* If the ARCHITECT iVancomycin assay results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
* Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
* Plasma samples from different anticoagulant tube types should not be used interchangeably for monitoring vancomycin. Use of citrate should be performed only when the blood is collected in a full tube so as not to incur a dilution effect.
* 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 that employ mouse monoclonal antibodies.[*10*](#unique_6_Connect_42_li_nf2_nft_dp)*,* [*11*](#unique_6_Connect_42_li_xls_gkt_1k)
* Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. The presence of heterophilic antibodies in a patient specimen may cause anomalous values to be observed. Additional information may be required for diagnosis.[*12*](#unique_6_Connect_42_li_jcz_gkt_1k)

# EXPECTED VALUES

Strong correlations have been shown between serum levels of vancomycin for both therapeutic and toxic effects. Therapeutic peak serum levels of 20 to 40 μg/mL and trough levels of
5 to 10 μg/mL have been reported to be effective for most strains of *staphylococci* and *streptococci*.[*4*](#unique_6_Connect_42_li_jy4_yft_dp) However, therapeutic levels of vancomycin must be individually established based on patient differences and bacterial susceptibility. The risk of toxicity is appreciably increased by high concentration or prolonged therapy in patients with renal insufficiency. Toxic effects, such as ototoxicity and nephrotoxicity, have resulted when serum concentrations of vancomycin reach 80 to 100 μg/mL and are rarely seen when serum levels are maintained below 30 μg/mL.[*13*](#unique_6_Connect_42_li_jq5_ggt_dp)*,* [*14*](#unique_6_Connect_42_li_e5f_hgt_dp) If an aminoglycoside is being used concurrently, the potential for toxicity is additive.[*4*](#unique_6_Connect_42_li_jy4_yft_dp)

Refer to drug manufacturer’s package insert for proper drug dosage and for vancomycin measurement sampling times.

For diagnostic purposes, the test findings should always be assessed in conjunction with the patient’s medical history, clinical examinations, and other findings.

# SPECIFIC PERFORMANCE CHARACTERISTICS

## Precision

The ARCHITECT iVancomycin assay is designed to have an assay precision of ≤ 10% total CV.

A study was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) Protocol EP5-A2.[*15*](#unique_6_Connect_42_li_otl_hgt_dp) Abbott Immunoassay-MCC (Liquid) (Levels 1, 2, and 3) and four human serum panels were assayed using two lots of reagents in replicates of three at two separate times per day for 20 days on two instruments. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized in the following table.\*

## Recovery

The ARCHITECT iVancomycin assay is designed to have a mean recovery of 100 ± 10%.

A study was performed on forty serum samples, where vancomycin was spiked into the samples to target concentrations of 3.2, 5, 7.5, 10, 15, 20, 30, and 45 μg/mL. The concentration of vancomycin was determined using the ARCHITECT iVancomycin assay and the resulting percent recovery was calculated. The overall percent recovery of the ARCHITECT iVancomycin assay was 100.2%. The percent recovery ranged from 98.0% to 105.4% for vancomycin concentrations from 5 to 45 μg/mL. The percent recovery was 86.4% for vancomycin concentration 3.2 μg/mL.\*

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

## Linearity

Based on guidance form the CLSI document EP6-A[*16*](#unique_6_Connect_42_li_jzs_l5d_fp), a study was performed to establish the linear range of the ARCHITECT iVancomycin assay.

## Sensitivity

The ARCHITECT iVancomycin assay is designed to have a Limit of Quantitation (LoQ) of ≤ 3.0 μg/mL. The LoQ is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ± 24%.

A study was performed based on guidance from the CLSI document EP17-A[*17*](#unique_6_Connect_42_li_yvr_hgt_dp) with four zero-level samples (normal human serum) and 8 samples with vancomycin concentrations ranging from 0.5 to 3.5 μg/mL. The samples were tested in at least 5 separate runs over a minimum of 3 days using 2 reagent lots and 2 instruments. In this study, the Limit of Blank (LoB) was 0.27 μg/mL, Limit of Detection (LoD) was 0.42 μg/mL and LoQ was 2.50 μg/mL.\*

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

## Specificity

Cross-reactivity was tested for compounds whose chemical structure or concurrent usage could cause potential interference with the ARCHITECT iVancomycin assay.

A study has demonstrated that vancomycin crystalline degradation product 1 (CDP-1) at a concentration of 10 μg/mL, has cross-reactivitya less than 0.42 μg/mL in the absence of vancomycin. CDP-1 at ≥ 5 μg/mL demonstrated cross-reactivity with samples containing vancomycin in the measurement range. CDP-1 may accumulate in patients with impaired renal function.[*18*](#unique_6_Connect_42_li_pnx_hgt_dp)*,* [*19*](#unique_6_Connect_42_li_ftc_3gt_dp)

The following compounds were tested in the absence of vancomycin after adding 500 μg/mL of each compound (except Methotrexate, Isoniazid and CDP-1) to human serum. Isoniazid was tested at 300 μg/mL. Methotrexate was tested at 227 μg/mL. Cross-reactivity of each compound was less than 0.42 μg/mL. The same compounds (except CDP-1 and Isoniazid) at the concentrations tested demonstrated no interference in the presence of vancomycin using the acceptance criteria of % recovery within 100 ± 10%.\* Interference was observed for CDP-1 and Isoniazid in the presence of vancomycin as follows:

* CDP-1 at ≥ 5 μg/mL interferes with samples containing vancomycin in the measurement range.
* Isoniazid at > 300 μg/mL interferes with samples containing vancomycin in the measurement range.

| Compounds Tested |
| --- |
| Acetaminophen | Isoniazid |
| Amikacin | Kanamycin B |
| Amphotericin B | Methotrexate |
| Ampicillin | Methylprednisolone |
| Caffeine | Naproxen |
| CDP-1 | Neomycin |
| Cephalexin | Nitrofurantoin |
| Cefotaxime | Penicillin G |
| Cephalothin | Penicillin V |
| Clindamycin | Prednisolone |
| Chloramphenicol | Rifampin |
| Chlorothiazide | Salicylic acid |
| Ciprofloxacin | Spectinomycin |
| Erythromycin | Streptomycin |
| Ethambutol | Sulfadiazine |
| 5-Fluorocytosine | Sulfamethoxazole |
| Furosemide | Tetracycline |
| Gentamicin | Ticarcillin |
| Heparin | Tobramycin |
| Hydrochlorothiazide | Trimethoprim |
| Ibuprofen |  |

a Cross-reactivity = Observed Test Concentration (μg/mL) – Control Concentration (μg/mL)

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

## Interference

Potential interference in the ARCHITECT iVancomycin assay from the following compounds is designed to have a mean recovery of 100 ± 10% of the control results at the levels indicated.

A study based on guidance from the CLSI Protocol EP7-A2[*20*](#unique_6_Connect_42_li_prh_3gt_dp) was performed for the ARCHITECT iVancomycin assay. Serum specimens with vancomycin levels from 4.4 to 48.8 μg/mL were supplemented with the following potentially interfering compounds. The mean recovery observed during the study ranged from 93.0% to 104.5%.\*

| Potentially Interfering Compound | Concentration |
| --- | --- |
| Triglycerides | 2500 mg/dL |
| Hemoglobin | 400 mg/dL |
| Bilirubin | 20 mg/dL |
| Low Protein | 3 g/dL |
| High Protein | 12 g/dL |
| HAMA | 1000 ng/mL |
| Rheumatoid Factor | 500 IU/mL |

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



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# BIBLIOGRAPHY

1. ABBOTT ARCHITECT i-Vancomycin package insert

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1. ABBOTT ARCHITECT i-Vancomycin Calibrator package insert

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1. Abbott ARCHITECT Operator’s Guide

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