ARCHITECT Folate

REF 1P74-25
REF 1P74-35

en

Folate
1P74
G5-6843/R06
B1P740

Read Highlighted Changes: Revised November 2015.

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.

WARNING:
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 which employ mouse monoclonal antibodies. These specimens should not be assayed with the ARCHITECT Folate assay. Refer to the **LIMITATIONS OF THE PROCEDURE** section in this package insert.

NAME

ARCHITECT Folate

INTENDED USE

The ARCHITECT Folate assay is a chemiluminescent microparticle Folate Binding Protein assay for the quantitative determination of folate in human serum, plasma, and red blood cells on the ARCHITECT iSystem.

SUMMARY AND EXPLANATION OF THE TEST

Folates are a class of vitamin compounds related to pteroylglutamic acid (PGA), which serve as cofactors in the enzymatic transfer of single carbon units in a variety of metabolic pathways.[1, 2] Folate mediated one-carbon metabolism represents one of the most important biochemical reactions that occur in cells. Folates are necessary for nucleic acid and mitochondrial protein synthesis, amino acid metabolism, and other cellular processes that involve single carbon transfers. Folates can serve as carbon donors or acceptors. Since different metabolic pathways require carbon groups with different levels of oxidation, cells contain numerous enzymes that change the oxidation state of carbon groups carried by folates[2] resulting in different metabolically active forms of folate. The predominant form of circulating folate is 5-methyltetrahydrofolic acid (5-mTHF). A methyl group is transferred from 5-mTHF to cobalamin in the pathway that links metabolism of folic acid and vitamin B12.[3]

Folate deficiency can be caused by low dietary intake, malabsorption due to gastrointestinal diseases, inadequate utilization due to enzyme deficiencies or folate antagonist therapy, drugs such as alcohol and oral contraceptives, and excessive folate demand, such as during pregnancy.[4] Because deficiencies of both vitamin B12 and folate can lead to megaloblastic (macrocytic) anemia, appropriate treatment requires differential diagnosis of the deficiency; thus, both vitamin B12 and folate values are needed. Low serum folate levels reflect the first stage of negative folate balance, and precede tissue depletion.[5] Low red-blood-cell folate values reflect the second stage of negative folate balance, and more closely correlate with tissue levels and megaloblastic anemia.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Folate assay is a two-step assay for the quantitative determination of folate in human serum, plasma, and red blood cells (RBC) using CMIA technology with flexible assay protocols, referred to as Chemiflex.

Two pre-treatment steps mediate the release of folate from endogenous folate binding protein.

1. In Pre-Treatment Step 1, sample and Pre-Treatment Reagent 2 (Dithiothreitol or DTT) are aspirated and dispensed into a reaction vessel (RV).
2. In Pre-Treatment Step 2, an aliquot of sample/Pre-Treatment Reagent 2 mixture is aspirated and dispensed into a second RV.
3. Pre-Treatment Reagent 1 (potassium hydroxide or KOH) is then added.
4. An aliquot of the pre-treated sample is transferred into a third RV, followed by the addition of Folate Binding Protein (FBP) coated paramagnetic microparticles and assay specific diluent. Folate present in the sample binds to the FBP coated microparticles.
5. After washing, pteroic acid-acridinium labeled conjugate is added and binds to unoccupied sites on the FBP-coated microparticles.
6. Pre-Trigger and Trigger Solutions are then added to the reaction mixture.
7. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of folate in the sample and the RLUs detected by the ARCHITECT iSystem optics.

In the Folate RBC assay, an initial manual pre-treatment step converts RBC-bound folate to measurable folate, after which these samples are processed as described above.

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

REAGENTS

Kit Contents

ARCHITECT Folate 1P74

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT iSystems. Please contact your local distributor.

REF	1P74-25	1P74-35
Σ	100	500
MICROPARTICLES	1 x 6.6 mL	1 x 27.0 mL
CONJUGATE	1 x 29.0 mL	1 x 29.0 mL
ASSAY SPECIFIC DILUENT	1 x 5.7 mL	1 x 25.3 mL
PRE-TREATMENT REAGENT 1	1 x 50.2 mL	1 x 50.2 mL
PRE-TREATMENT REAGENT 2	1 x 6.6 mL	1 x 27.0 mL
SPECIMEN DILUENT	1 x 5.5 mL	1 x 25.9 mL

MICROPARTICLES Anti-Folate Binding Protein (mouse, monoclonal) coupled to microparticles affinity-bound with Folate Binding Protein (bovine), in TRIS buffer with protein stabilizers (human serum albumin and caprine). Minimum concentration: 0.08% solids. Preservatives: sodium azide and antimicrobial agents.

CONJUGATE Pteroic Acid (PTA) - acridinium labeled conjugate in MES buffer with protein stabilizer (porcine). Minimum concentration: 4 ng/mL. Preservative: antimicrobial agents.

ASSAY SPECIFIC DILUENT Folate Assay Specific Diluent containing borate buffer. Preservatives: sodium azide and antimicrobial agents.

PRE-TREATMENT REAGENT 1 Folate Pre-Treatment Reagent 1 containing potassium hydroxide.

REF	1P74-25	1P74-35
Σ	100	500

PRE-TREATMENT REAGENT 2 Folate Pre-Treatment Reagent 2 containing dithiothreitol (DTT) in acetic acid buffer with EDTA.

SPECIMEN DILUENT Folate Specimen Diluent containing TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.

Other Assay-Specific Reagents

MANUAL DILUENT 1 x 4 mL ARCHITECT Folate Manual Diluent, **REF** 1P74-50, containing TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.

RBC LYSIS DILUENT 1 x 12.5 mL ARCHITECT Folate RBC Lysis Diluent, **REF** 1P74-40. Folate RBC Lysis Diluent (L2) containing citric acid and guanidine hydrochloride. Preservative: antimicrobial agent.

LYSIS REAGENT 4 x 285-385 mg Folate Lysis Reagent, **REF** 3P21-60. Folate Lysis Reagent (L1) containing ascorbic acid and guanidine hydrochloride.

Other Reagents

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

NOTE: Bottle and volume varies based on order.

Warnings and Precautions

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

Safety Precautions

CAUTION: This product contains human-sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[6-9]

The human serum albumin used in the Microparticles and Specimen Diluent has been tested and found to be nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.

The following warnings and precautions apply to: **ASSAY SPECIFIC DILUENT**

DANGER:	Contains disodium tetraborate, anhydrous and sodium azide.
H360	May damage fertility or the unborn child.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P201	Obtain special instructions before use.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P308+P313	IF exposed or concerned: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: **PRE-TREATMENT REAGENT 1**

DANGER:	Contains potassium hydroxide.
H314	Causes severe skin burns and eye damage.
H290	May be corrosive to metals.
Prevention	
P234	Keep only in original container.
P260	Do not breathe mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P301+P330+P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water / shower.
P310	Immediately call a POISON CENTER or doctor / physician.
P390	Absorb spillage to prevent material damage.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: **MICROPARTICLES** / **SPECIMEN DILUENT**

Contains sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
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 reagents, calibrators, or controls 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.**

- **Prolonged exposure of Folate Pre-Treatment Reagent 1 to air without septum in place may compromise performance.**
  - 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

NOTE: Hereafter, instructions in this package insert that pertain ONLY to the Folate RBC assay are contained in a text box.

When stored and handled as directed the ARCHITECT Folate Reagent Kit, Folate RBC Lysis Diluent, Folate Manual Diluent, and the Controls 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. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

NOTE: The ARCHITECT Folate Reagent Kit is shipped cold and should be stored at 2-8˚C after receipt. Calibrators are shipped frozen and must be stored at -10˚C or colder.

Calibrators and Controls are sensitive to light. **Store bottles in carton to protect from light.**

Unreconstituted Folate Lysis Reagent (L1) must be stored at 15-30˚C. Reconstituted Folate Lysis Reagent (L1) must be stored at 2-8˚C. The expiration date is 7 days from the date of reconstitution. Write the expiration date of the reconstituted Folate Lysis Reagent (L1) on the bottle but do not exceed the lot expiration date printed on the bottle.

* 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 Folate (1P74) assay files are named “Folate II” and “FolateRBC”.
- The ARCHITECT Folate II (assay number 685) and/or FolateRBC (assay number 686) assay file(s) must be installed on the ARCHITECT iSystem 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.
- ARCHITECT maintenance procedure *6041 Daily Maintenance* (version 5 or higher) must be installed on the ARCHITECT iSystem prior to performing the assay. For information on installing and deleting maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 2.
- ARCHITECT maintenance procedure *6041 Daily Maintenance* (version 5 or higher) must be run at a minimum once every 24 hours. For laboratories processing a higher volume of B12 (List 6C09) and Folate tests on a single module, this procedure must be run more than once in a 24-hour period.
  - If B12 (List 6C09) and Folate are run on a single module and you run > 100 B12 (List 6C09) or > 100 Folate tests in 24 hours, perform the *6041 Daily Maintenance* procedure (version 5 or higher) after every 100 B12 (List 6C09) or 100 Folate tests run.
  - Refer to **LIMITATIONS OF THE PROCEDURE** for additional information.
- If microbial contamination is suspected when running ARCHITECT Folate on the ARCHITECT iSystem due to shifts in results and/or the incidence of calibration failures with the following error codes:
  - 1402 - Assay (Folate II/FolateRBC). Number (685/686) Calibration failure, calibrators incorrectly loaded
  - 1206 - Assay (Folate II/FolateRBC). Number (685/686) Calibration failure, concentration too high for Cal A
  - 1120 - Assay (Folate II/FolateRBC). Number (685/686) Calibration failure, fit response too low for Cal A

  the following actions must be taken to protect the integrity of assay results:
  - Contact your local customer support representative to schedule the local Abbott Service Representative to perform the *2180 Internal Decontamination* procedure on your ARCHITECT iSystem. If the instrument is connected to an Automatic Reconstitution Module (ARM), the *2182 ARM Decontamination* procedure must also be executed.
  - It may be necessary to repeat the decontamination procedure if microbial contamination recurs.

- When configuring the host for the Folate RBC assay, set the appropriate default dilutions:
  - If running whole blood specimens or whole blood controls, configure the default dilution as “RBC DIL”.
  - If running controls other than whole blood controls, configure the default dilution as “UNDILUTED”.

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 concentration 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	2.265	nmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with the ARCHITECT Folate assays:

Folate	Folate RBC
Serum (glass or plastic tube) Serum separator (SST) Lithium heparin plasma Lithium heparin plasma separator (PST)	Whole blood dipotassium EDTA (K_2 EDTA) Whole blood tripotassium EDTA (K_3 EDTA)

- Other specimen collection tube types have not been tested with these assays.
- **Do not use human plasma collected in dipotassium or tripotassium EDTA tubes for Folate.**

- **Do not use human whole blood collected in lithium heparin tubes for Folate RBC.**

- Performance has not been established for the use of cadaveric specimens or body fluids other than human serum, plasma, and whole blood.
- 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 ARCHITECT Folate assays.
- Human serum, plasma, or whole blood specimens to be tested for folate should be protected from light.[10, 11]
- Serum or plasma specimens should be collected from fasting individuals. Recent food intake may appreciably increase the folate concentration.[11]
- Do not use hemolyzed specimens. Serum or plasma specimens that are hemolyzed will give falsely elevated folate levels.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - 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.

  Serum or plasma specimens containing red blood cells may give falsely elevated folate levels.
- 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

- Human serum, plasma, or whole blood specimens to be tested for folate should be protected from light.[10, 11]
- Remove serum from clot or separator gel as soon as possible after complete clot formation. If testing will not be performed immediately, serum specimens may be stored either at 2-8°C for up to 7 days or frozen (-10°C or colder) for up to 30 days prior to being tested.
- Remove plasma from red blood cells as soon as possible upon receipt.[11] If testing will not be performed immediately, plasma specimens may be stored either at 2-8°C for up to 7 days or frozen (-10°C or colder) for up to 30 days prior to being tested.
- Avoid more than 3 freeze/thaw cycles.

- For red blood cell folate measurements, mix whole blood tube by inverting 10 times to ensure a homogeneous sample. **Determine the hematocrit of each specimen prior to storage.** The hematocrit value will be required in Calculations 1 and 2 beginning on page 6.
- If testing will not be performed immediately, whole blood specimens may be stored at 2-8°C for up to 2 days or frozen (-10°C or colder) for up to 30 days prior to being tested.
- Avoid more than 1 freeze/thaw cycle.

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

1P74 ARCHITECT Folate Reagent Kit

Materials Required but not Provided

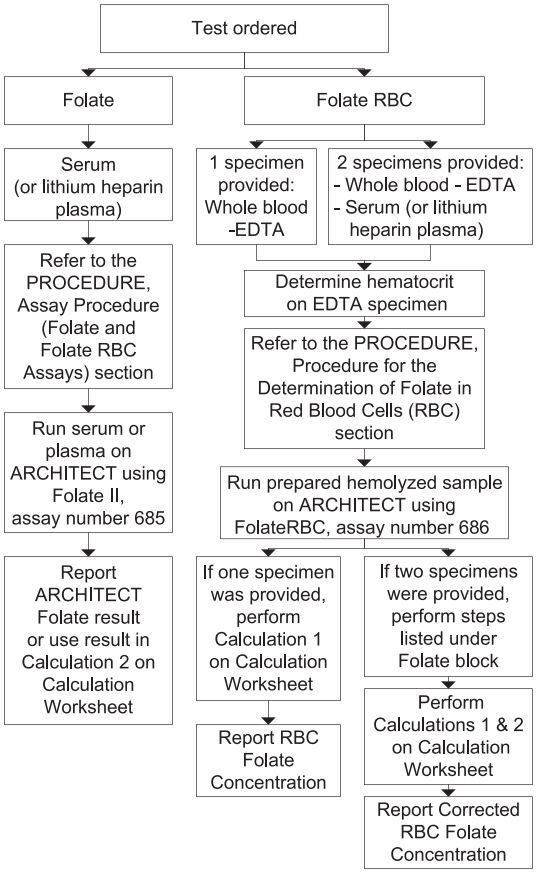
- ARCHITECT Folate Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 1P74-01 ARCHITECT Folate Calibrators
- 1P74-10 ARCHITECT Folate Controls
- 1P74-50 ARCHITECT Folate Manual Diluent
- 1P74-40 ARCHITECT Folate RBC Lysis Diluent
- 3P21-60 Folate Lysis Reagent
- 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.

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

Assay Procedure Overview

The Folate result is obtained using serum or plasma specimens. The Folate RBC result is obtained using a hemolysate prepared from whole blood. The Folate RBC result includes folate present in the RBCs and in the plasma. In order to obtain the folate concentration only in the RBCs, both specimens are required and a calculation is performed using results from both assays to obtain a Corrected RBC Folate result (if desired). The three paths are shown in the flowchart below based on the specimens provided.

NOTE: The ARCHITECT Folate (1P74) assay files are named "Folate II" and "FolateRBC".



Procedure for the Determination of Folate in Red Blood Cells (RBC)

NOTE: Determine hematocrit of EDTA specimen. This value will be required in Calculations 1 and 2 beginning on page 6.

Part 1: Reconstitution of Folate Lysis Reagent (L1)

- Reconstitute one bottle of the Folate Lysis Reagent (L1) by adding 30 mL distilled or deionized water.
- Cap the reagent bottle and mix by inverting 10 times and let stand for 15 minutes.
- The expiration date is 7 days from the date of reconstitution. Write the expiration date of the reconstituted Folate Lysis Reagent (L1) on the line provided on the bottle label, but do not exceed the lot expiration date printed on the bottle. Store at 2-8°C when not in use.

Part 2: Preparation of Red Blood Cell Hemolysate

NOTE: The assay must be initiated on the final hemolysate within 2 hours.

- Invert the reconstituted Folate Lysis Reagent (L1) an additional 10 times. Pipette 1.0 mL into a suitable sample tube with a cap (example: 2 mL tube).
- Mix whole blood tube by inverting 10 times to ensure a homogeneous sample.
- Add 100 µL of whole blood sample to the sample tube containing the 1.0 mL of the reconstituted Folate Lysis Reagent (L1).
- Cap the tube and mix by inverting 10 times or vortexing and allow to stand at room temperature (15-30°C) for 90 minutes (± 5 minutes). **Protect from light.**
- Pipette 100 µL ARCHITECT Folate RBC Lysis Diluent (L2) into a new sample tube (or ARCHITECT sample cup). Then add 100 µL of hemolyzed sample.
- Mix by swirling or vortexing and initiate assay on this sample within 2 hours.

Assay Procedure (Folate and Folate RBC Assays)

- 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.
  - **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, discard the cap and 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. Refer to ARCHITECT Operations Manual, Section 5, for details on how to load reagents.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.

  NOTE: The ARCHITECT Folate (1P74) assay files are named “Folate II” and “FolateRBC”.
- Select the appropriate assay protocol.
  - If running a serum or plasma specimen/control, select Folate II (assay number 685, “UNDILUTED”).
  - If running an automated dilution on a serum or plasma specimen, select the 1:2 protocol of Folate II (assay number 685, “1:2”).

- If running a whole blood specimen or whole blood control, select FolateRBC (assay number 686, “RBC DIL”).
- If running controls other than whole blood controls with the FolateRBC assay, select the undiluted protocol of FolateRBC (assay number 686, “UNDILUTED”).

- For additional information on ordering patient specimens, calibrators, 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: 85 µL
  - Sample volume for each additional test from same sample cup: 35 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 35 µL
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

- Prepare ARCHITECT Folate Calibrators and Controls.
  - Mix calibrator(s) and controls by gentle inversion (3-5 times) before use.
  - Discard ARCHITECT Folate Calibrators after 3 freeze/thaw cycles.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    for each calibrator: 6 drops

    for each control: 6 drops

  NOTE: It is very important to return the ARCHITECT Folate Calibrators and Controls to their carton and correct storage conditions immediately after use, as follows.
  - **Store ARCHITECT Folate Calibrators at -10°C or colder.**
  - **Store ARCHITECT Folate Controls at 2-8°C.**
- 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 (for folate serum or plasma determinations only)

Specimens with a folate serum or plasma value exceeding 20.0 ng/mL are flagged with the code "> 20.0" and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

If using the Automated Dilution Protocol (assay number 685, 1:2 Protocol), the system performs a 1:2 dilution. The system will use the dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result.

Manual Dilution Procedure

Suggested dilution: 1:2

It is recommended that dilutions not exceed 1:4.

1. For a 1:2 dilution, add 100 µL of the patient specimen to 100 µL of ARCHITECT Folate Manual Diluent (1P74-50).

   For a 1:4 dilution, add 100 µL of the patient specimen to 300 µL of ARCHITECT Folate Manual Diluent (1P74-50).
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. This will be the reported result.

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

Calibration

- Separate calibrations are required for ARCHITECT Folate II and ARCHITECT FolateRBC assay files.
- Test Calibrators A-F in duplicate. The calibrators should be priority loaded.

  A single sample of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0.0 - 20.0 ng/mL.
- Once an ARCHITECT Folate 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 best results, establish statistically-based QC ranges to monitor and control the frequency of recalibration.
- 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 Folate 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.

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

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 Folate assay belongs to method group 1.

RESULTS

Calculation

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

Alternate Result Units

- The default result unit for the ARCHITECT Folate assay is ng/mL. When the alternate result unit, nmol/L, is selected, the conversion factor used by the system is 2.265.
- Conversion Formula: (concentration in ng/mL) x (2.265) = nmol/L
- Formulas and examples indicate ng/mL as the result unit. If the chosen ARCHITECT Folate result is nmol/L, the final result would be in nmol/L.

Calculation of Red Blood Cell Folate Concentration (for Folate RBC Assay only:

Calculation performed by the ARCHITECT iSystem

When the FolateRBC assay is used (assay number 686 utilizing "RBC DIL" protocol), the ARCHITECT iSystem automatically corrects the reported sample result for dilutions that were required during the preparation of the red blood cell hemolysate. This is the ARCHITECT FolateRBC test result. **Do not report this result. Further calculation is required.**

NOTE: A Calculation Worksheet is provided at the end of this package insert to assist with RBC Folate calculations.

Calculations performed by the Operator

Calculation 1

To calculate the RBC Folate concentration from the ARCHITECT FolateRBC test result, use the following formula:

$$\text{RBC Folate Concentration (ng/mL)} = \frac{A}{B} \times 100$$

where:

A = ARCHITECT FolateRBC test result (ng/mL)

B = % Hematocrit (value obtained prior to storage or prior to Procedure for Folate RBC)

Example:

ARCHITECT FolateRBC test result = 64.0 ng/mL

% Hematocrit = 32

$$\text{RBC Folate Conc.} = \frac{64.0 \text{ ng/mL}}{32} \times 100 = 200.0 \text{ ng/mL}$$

Calculation 2

Calculation of Corrected Red Blood Cell Folate Concentration (for Folate RBC Assay only):

Folate concentrations from serum or plasma are very small as compared to RBC folate concentrations, in most cases. It is possible for the serum or plasma folate concentration to be within or above the expected normal range while the RBC folate concentration is below the expected normal range. In these instances, a correction may be needed. The Folate serum (or plasma) result is required for this calculation. The following calculation will correct for serum or plasma folate concentrations:

Corrected RBC Folate Conc. (ng/mL) =

$$C - \left[D \times \left[\frac{100 - B}{B} \right] \right]$$

where:

B = % Hematocrit (value used for B in Calculation 1)

C = RBC Folate Concentration result from Calculation 1 (ng/mL)

D = ARCHITECT Folate Serum (or plasma) result (ng/mL)

Example:

% Hematocrit = 32

RBC Folate Concentration result = 200 ng/mL

ARCHITECT Folate Serum (or plasma) result = 25.0 ng/mL

Corrected RBC Folate Conc. =

$$200.0 \text{ ng/mL} - \left[25.0 \text{ ng/mL} \times \left[\frac{100 - 32}{32} \right] \right] = 146.9 \text{ ng/mL}$$

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

The measuring interval of the ARCHITECT Folate assay is 1.5 ng/mL to 20.0 ng/mL.

LIMITATIONS OF THE PROCEDURE

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the folate level is inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- 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 Folate that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[12, 13]
- 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.[14]
- Serum or plasma containing red blood cells may give falsely elevated folate levels. These samples should be centrifuged prior to use. Serum or plasma samples that are hemolyzed will give falsely elevated folate levels.
- Serum and plasma specimens from patients with renal impairment or failure (including dialysis patients) may exhibit varying degrees of falsely depressed folate values.[15] Therefore, to evaluate folate patients with renal impairment or failure, it is recommended that low ARCHITECT Folate values be confirmed by an alternate folate method such as the ARCHITECT Folate RBC assay.
- Methotrexate, aminopterin, and folinic acid (Leucovorin) are chemotherapeutic agents whose molecular structures are similar to folate. These agents cross react with folate binding protein in folate assays.[16]
- Samples to be tested for folate should be protected from light. Light accelerates the degradation of folate.
- Accumulation of denatured protein from the pre-treatment step in the sample probe may impact results of other assays on the ARCHITECT iSystem. ARCHITECT maintenance procedure *6041 Daily Maintenance* (version 5 or higher) must be run to eliminate this effect. Refer to the **INSTRUMENT PROCEDURE** section for instructions.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal and deficient ranges, which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) document C28-A3.[17] The nutritional status of the specimen donors was unknown. All specimens tested were from fasting, apparently healthy males and non-pregnant females greater than 18 years old from a UK population. Serum and whole blood samples were tested for serum/plasma and red blood cell folate using the ARCHITECT Folate assay. Data from this study are summarized in the following table.

Expected Values Data Statistics ng/mL (nmol/L)

	n	Min	Max	Expected Values
Serum/Plasma	155	1.6 (3.6)	19.5 (44.2)	3.1 - 20.5 (7.0 - 46.4)
Whole Blood	168	58.5 (132.5)	733.1 (1660.5)	126.0 - 651.1 (285.4 - 1474.7)

Folate Deficients/Indeterminates

- Folate deficiency is typically associated with serum levels less than 3.5 ng/mL or RBC levels less than 150 ng/mL.[18, 21]
- Patients with RBC folate levels ranging from 150 to 250 ng/mL have been associated with megaloblastic erythropoiesis, but folate values in patients with normal erythropoiesis can also fall within this range.[18]
- Often, the diagnosis of folate deficiency cannot be based solely on serum or RBC folate levels, and further testing may be required.[18-20]

SPECIFIC PERFORMANCE CHARACTERISTICS

Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT Folate assay is designed to have a within-laboratory imprecision of:

- ≤ 12% total CV for serum samples from 3.5 ng/mL to 20 ng/mL and ≤ 11% CV for RBC hemolysate between 150 ng/mL and 640 ng/mL.
- a Standard Deviation (SD) ≤ 0.42 for serum samples below 3.5 ng/mL and SD ≤ 16.50 for RBC hemolysate samples below 150 ng/mL.

A study was performed based on guidance from the CLSI document EP5-A2.[22] Three serum panels (S1, S2, and S3) and three hemolysate panels (H1, H2, and H3) were assayed, using 1 instrument, in replicates of 3, at two separate times per day for 20 days, using 2 lots of reagents. Data from this study are summarized in the following table.

ARCHITECT Folate Within-Laboratory Precision

Sample Level	Reagent Lot	Mean ng/mL (nmol/L)	Within Run		Total	
			SD ng/mL (nmol/L)	%CV	SD ng/mL (nmol/L)	%CV
S1	1	3.5 (7.9)	0.12 (0.27)	3.5	0.14 (0.32)	3.9
	2	3.6 (8.2)	0.14 (0.32)	3.9	0.17 (0.39)	4.7
S2	1	10.8 (24.5)	0.18 (0.41)	1.7	0.41 (0.93)	3.8
	2	11.2 (25.4)	0.21 (0.48)	1.9	0.44 (1.00)	4.0
S3	1	16.8 (38.1)	0.27 (0.61)	1.6	0.53 (1.20)	3.1
	2	17.0 (38.5)	0.24 (0.54)	1.4	0.61 (1.38)	3.6
H1	1	113.2 (256.4)	6.10 (13.82)	5.4	8.82 (19.98)	7.8
	2	118.1 (267.5)	4.69 (10.62)	4.0	6.49 (14.70)	5.5
H2	1	222.9 (504.9)	7.04 (15.95)	3.2	13.29 (30.10)	6.0
	2	221.9 (502.6)	5.59 (12.66)	2.5	12.19 (27.61)	5.5
H3	1	367.2 (831.7)	7.87 (17.83)	2.1	21.60 (48.92)	5.9
	2	359.1 (813.4)	8.90 (20.16)	2.5	22.97 (52.03)	6.4

Autodilution Verification

The ARCHITECT Folate assay was designed to have an absolute mean change in measured concentration of ≤ 20% when comparing manual to autodilution (1:2 dilution). The assay was evaluated for autodilution with the 1:2 autodilution method vs. the 1:2 and 1:4 manual dilution methods using 18 specimens with folate values ranging from 20 to 40 ng/mL. Three replicates each of the autodiluted and manually diluted samples were assayed on one instrument using the ARCHITECT Folate assay. Data from this study are summarized in the following table.

Percent Differences Across Samples

Dilution Comparison	n	Mean/Median %Difference
Auto (1:2) vs. Manual (1:2)	18	0.8
Auto (1:2) vs. Manual (1:4)	18	5.9

Linearity

The ARCHITECT Folate assay was evaluated for linearity by mixing a high (> 20 ng/mL) serum specimen pool in specific ratios with a low (≤ 3.5 ng/mL) serum specimen pool to create 11 mixed sample pools. All pools were tested by the ARCHITECT Folate assay. Based on guidance from CLSI document EP6-A[23], the study demonstrated linearity from 1.6 to 20 ng/mL.

Accuracy to World Health Organization (WHO) Standard

The ARCHITECT Folate assay was evaluated for bias relative to the Folate WHO International Standard 03/178. A minimum of 38 replicates of the WHO Standard was tested on each of 2 instruments. A different reagent lot was used on each instrument and one calibrator lot was used for both instruments.

The Folate assay results were accurate within ± 10% to the 1st International Reference Standard (I.S.) for Serum Folate (03/178). Data from this study are summarized in the following table.

n	Median ng/mL (nmol/L)	Target ng/mL (nmol/L)	Diff.[a] ng/mL (nmol/L)	Two-Sided 95%CL[b] ng/mL (nmol/L)	%Diff.[a]	Two-Sided 95%CL[b] %Diff.[a]
76	5.4 (12.2)	5.3 (12.0)	0.1 (0.2)	0.0, 0.1 (0.0, 0.2)	1.3	-0.6, 1.3

[a] Diff. = Difference

[b] CL = Confidence Limit

Sensitivity

Sensitivity is defined as the Limit of Quantitation (LoQ), which is the lowest amount of analyte in a sample that can be accurately quantitated with a Total Error of ± 39%.[24]

The ARCHITECT Folate assay is designed to have an LoQ of ≤ 3.5 ng/mL.

The Limit of Blank (LoB), Limit of Detection (LoD), and LoQ of the ARCHITECT Folate assay were determined based on guidance from CLSI document EP-17A[25], using proportions of false positives (α) less than 5% and false negatives (β) less than 5%. These determinations were performed using 1 zero-level (3 replicates) and 5 low-level folate samples (3 replicates each). The following values were determined in this study: LoB = 0.3 ng/mL (0.7 nmol/L), LoD = 0.5 ng/mL (1.1 nmol/L), and LoQ = 1.5 ng/mL (3.4 nmol/L).

Specificity

The specificity of the ARCHITECT Folate assay was evaluated by testing cross-reactivity with aminopterin, folinic acid, and methotrexate in processed human serum containing endogenous folate. Therapeutic levels of these drugs can greatly exceed the levels tested in this study and are expected to interfere with the ARCHITECT Folate assay.[16] A study was performed with the ARCHITECT Folate assay based on guidance from the CLSI document EP7-A2.[26] Aliquots of human serum at two different folate concentrations were supplemented with potential cross-reactants and tested for folate. Data from this study are summarized in the following table.

Interferent	Reference		Test		Diff.[a] ng/mL	%Diff.[b]	%Cross-Reactivity[c]
	n	Mean/Median ng/mL	n	Mean/Median ng/mL			
Aminopterin ≥ 500 ng/mL	40	2.6	40	8.3	5.7	219.2	1.1
	40	7.4	39	13.0	5.6	75.7	1.1
Folinic Acid ≥ 100 ng/mL	40	2.9	40	3.4	0.5	17.2	0.5
	40	7.9	44	7.3	-0.6	-7.4	-0.6
Methotrexate ≥ 100 ng/mL	45	2.7	40	4.8	2.1	77.8	2.1
	40	7.6	40	8.9	1.4	18.2	1.4

[a] Difference = test mean [or median] conc. - reference mean [or median] conc.

[b] % Difference = Difference / reference mean [or median] conc. x 100

[c] % Cross-Reactivity = Difference / interferent conc. x 100

Interference

Potential interference in the ARCHITECT Folate assay from bilirubin, (conjugated and unconjugated), triglycerides, and protein was demonstrated in a study based on guidance from CLSI document EP7-A2.[26] Hemoglobin was not tested due to the high folate content in red blood cells. Refer to the **LIMITATIONS OF THE PROCEDURE** section. Data from this study are summarized in the following table.

Interferent	Reference		Test		Diff.[a] ng/mL	%Diff.[b]
	n	Mean/Median ng/mL	n	Mean/Median ng/mL		
Bilirubin (unconjugated) ≤ 20 mg/dL	40	2.1	40	2.0	-0.1	-4.0
	40	7.9	40	7.6	-0.3	-3.8
Bilirubin (conjugated) ≤ 20 mg/dL	40	1.8	40	1.7	-0.1	-5.6
	40	7.5	40	7.0	-0.5	-6.7
Protein ≤ 12 g/dL	40	2.6	40	2.9	0.3	11.5
	40	8.8	40	9.1	0.3	2.8
Triglycerides ≤ 3000 mg/dL	40	2.1	40	2.2	0.1	4.8
	40	7.9	39	8.0	0.1	1.8

[a] Difference = test mean [or median] - reference mean [or median]

[b] % Difference = Difference / reference mean [or median] x 100

Tube Type Matrix Comparison

The following tube types are acceptable for use with the ARCHITECT Folate assay:

- Glass: Serum
- Plastic: Serum, Serum Separator Tube (SST), Lithium Heparin Plasma Tube, and Lithium Heparin Plasma Separator Tube (PST),

On average, the tube types evaluated showed less than a 10% difference when compared to the control tube type (plastic serum). The distribution of the percent differences per tube type is listed in the following table.

	Distribution of Absolute %Differences[a]	
Evaluation Tube Type	**< 10%**	**10% to 20%**
Glass, Serum	92.6% (25/27)	7.4% (2/27)
Serum Separator Tube, Plastic (SST)	100.0% (27/27)	0.0% (0/27)
Lithium Heparin Plasma Tube	80.0% (20/25)	20.0% (5/25)
Lithium Heparin Plasma Separator Tube (PST)	92.6% (25/27)	7.4% (2/27)

[a] There were no absolute % difference values > 20%.

The following tube types are acceptable for use with the ARCHITECT Folate RBC assay:

- whole blood dipotassium EDTA (K_2 EDTA)
- whole blood tripotassium EDTA (K_3 EDTA)

All K3 EDTA tubes evaluated (n=27) showed less than 10% difference when compared to matched K2 EDTA tubes.

Method Comparison

Two correlation studies were performed based on guidance from CLSI document EP9-A2[27] using the Passing-Bablok[28] regression method to compare the ARCHITECT Folate assay to the AxSYM Folate assay. One study was performed with serum/plasma specimens and the other with whole blood specimens. The analysis of the results from the serum/ plasma study included both the full range of specimens analyzed and a truncated range for the AxSYM Folate assay. The truncated range minimizes any effects due to apparent non-linearity of AxSYM results at the clinically less significant higher folate concentrations. Truncation was unnecessary for whole blood specimens. The tables below summarize the results of these correlation analyses.

Correlation of ARCHITECT Folate to AxSYM Folate

	Conc. Range ng/mL (nmol/L)			Intercept ng/mL	
Specimen Type	**ARCHITECT**	**AxSYM**	**r[a]**	**(nmol/L)**	**Slope**
Serum/Plasma (n=144)	0.9-28.9 (2.0-65.5)	2.2-33.4 (5.0-75.7)	0.921	-5.85 (-13.25)	1.27
Truncated Serum/Plasma (n=43)	0.9-12.6 (2.0-28.5)	2.2-14.0 (5.0-31.7)	0.963	-1.19 (-2.70)	0.82
Whole Blood (n=123)	145.5-1014.6 (329.6-2298.1)	155.8-1034.5 (352.9-2343.1)	0.895	-33.36 (-75.56)	0.74

[a] r = Correlation Coefficient

Some serum and plasma samples in the upper region of the dynamic range may read lower in the AxSYM Folate assay when compared to the ARCHITECT Folate assay. This can result in a decreased correlation coefficient value over the entire measurement range.

Two correlation studies were performed using the Passing-Bablok regression method to compare the ARCHITECT Folate assay to the ARCHITECT Folate Non-US assay. One study was performed with serum/ plasma specimens and the other with whole blood specimens. The table below summarizes the results of these correlation studies.

Correlation of ARCHITECT Folate to ARCHITECT Folate Non-US

	Conc. Range ng/mL (nmol/L)			Intercept ng/mL	
Specimen Type	**ARCHITECT**	**ARCHITECT Non-US**	**r[a]**	**(nmol/L)**	**Slope**
Serum/Plasma (n=140)	1.4-28.9 (3.2-65.5)	1.0-31.3 (2.3-70.9)	0.997	-0.38 (-0.86)	0.98
Whole Blood (n=131)	145.5-1014.6 (329.6-2298.1)	97.8-900.5 (221.5-2039.6)	0.973	55.67 (126.09)	0.89

[a] r = Correlation Coefficient

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Key to Symbols

	Caution
	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ASSAY SPECIFIC DILUENT	Assay Specific Diluent
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
DANGER: REPRODUCTIVE HAZARD	Danger: Reproductive Hazard
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
LYSIS REAGENT	Lysis Reagent
MANUAL DILUENT	Manual Diluent
MICROPARTICLES	Microparticles
PRE-TREATMENT REAGENT 1	Pre-Treatment Reagent 1
PRE-TREATMENT REAGENT 2	Pre-Treatment Reagent 2
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCT OF IRELAND	Product of Ireland
RBC LYSIS DILUENT	RBC Lysis Diluent
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
SPECIMEN DILUENT	Specimen Diluent
TRIGGER SOLUTION	Trigger Solution
WASH BUFFER	Wash Buffer

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Calculation Worksheet (for RBC Folate Calculations)

Sample ID_____

Date_____

Initials_____

Notes **The ARCHITECT Folate (1P74) assay files are named: "Folate II" and "FolateRBC".**

If only a whole blood specimen was provided for the Folate RBC test, perform Calculation 1.

If the Corrected RBC Folate Concentration result is desired and both a whole blood specimen and a serum or plasma specimen were provided, perform Calculations 1 and 2. (See flowchart in Assay Procedure Overview section.)

Calculation 1

Calculate the RBC Folate Concentration.

Step 1. Record values.

A = ARCHITECT FolateRBC test result (ng/mL) (value reported by ARCHITECT) _____ (A)

B = % Hematocrit (value obtained in the Storage section on page 4) _____ (B)

Step 2. Perform calculation.

$$\textbf{RBC Folate Concentration (ng/mL)} = \frac{A}{B} \times 100 = \text{(C)}$$

This is the RBC Folate Concentration result. (C)

If the Corrected RBC Folate Concentration result is desired, perform Calculation 2 to correct for the serum (or plasma) Folate concentration. (The Folate serum (or plasma) result is required for this calculation.)

Calculation 2

Calculate the Corrected RBC Folate Concentration.

Step 1. Record values.

B = % Hematocrit (value used for B in Calculation 1) _____ (B)

C = RBC Folate Concentration result from Calculation 1 (ng/mL) _____ (C)

D = ARCHITECT Folate Serum (or plasma) test result (ng/mL) _____ (D)

Step 2. Perform calculation by following the steps listed below the equation.

$$\textbf{Corrected RBC Folate Conc. (ng/mL)} = C - \left[D \times \left[\frac{100 - B}{B} \right] \right]$$

Subtract B from 100. _____ (E)

Divide result obtained in (E) by B. _____ (F)

Multiply result obtained in (F) by D. _____ (G)

Subtract (G) from C. _____ (H)

This is the Corrected RBC Folate Concentration result (H).