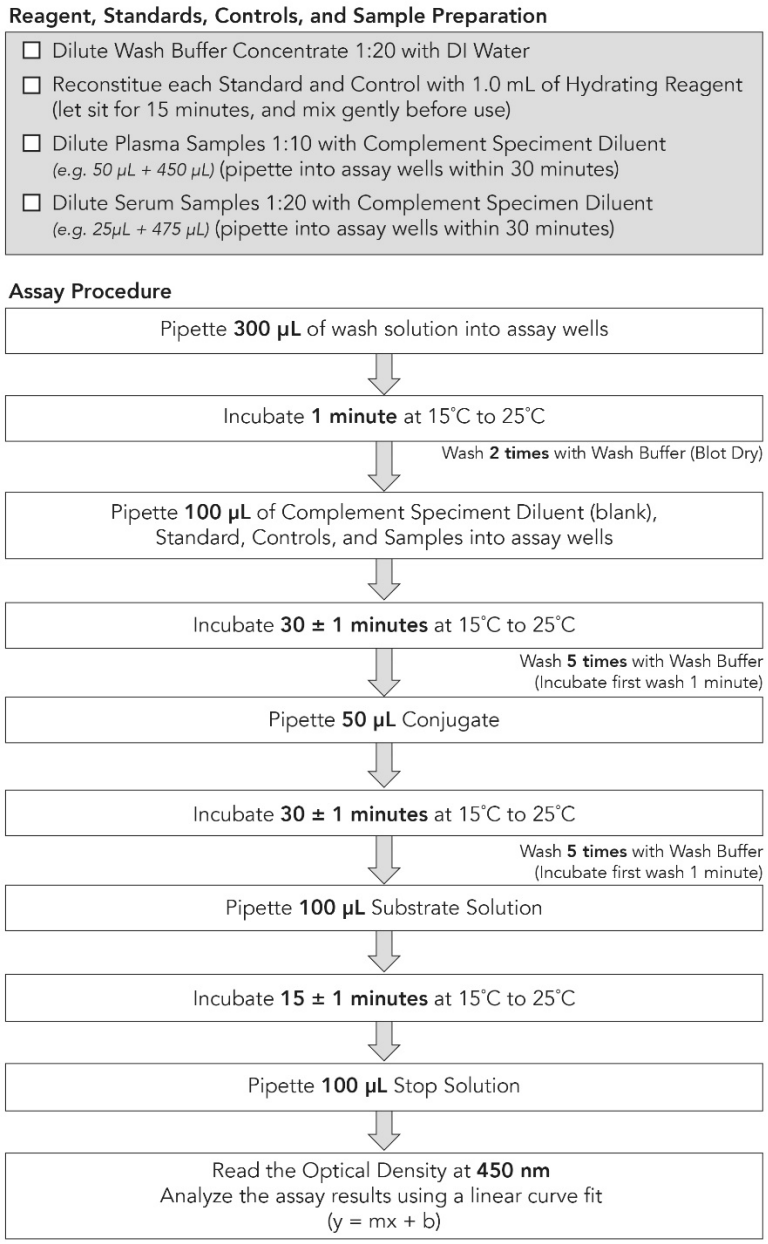
QUIDEL

MicroVue™ Complement
Bb Plus Fragment EIA

An enzyme immunoassay for quantitation of the complement fragment Bb, an activation fragment of Factor B of the alternative pathway of complement, in human plasma or serum

SUMMARY



INTENDED USE

The MicroVue Bb Plus EIA kit measures the amount of the complement fragment Bb, an activation fragment of Factor B of the alternative pathway of complement, in human plasma or serum. Measurement of Bb in human plasma or serum provides evidence of the involvement of the alternative pathway of complement. Measurement of alternative pathway activation aids in the diagnosis of several kidney diseases, e.g., chronic glomerulonephritis, lupus nephritis, as well as several skin diseases, e.g., dermatitis herpetiformis and pemphigus vulgaris. Other diseases in which activation of the alternative pathway of complement has been observed include rheumatoid arthritis, sickle cell anemia, and gram-negative bacterial infections.

SUMMARY AND EXPLANATION

The alternative complement pathway provides innate protection against microbial agents in the absence of specific antibody.[1-5] The activation of this complement pathway can be triggered by a variety of substances including microbial polysaccharides or lipids, gram-negative bacterial lipopolysaccharides, and surface determinants present on some viruses, parasites, virally infected mammalian cells, and cancer cells. In autoimmune diseases, the alternative complement pathway may contribute directly to tissue damage.

A centrally important reaction that occurs during alternative pathway activation is the conversion of the 93 Kd molecular weight Factor B zymogen to an active proteolytic enzyme. This is accomplished in a two-step reaction. During the first reaction step the Factor B forms a magnesium-dependent complex with C3(H20) or C3b.[4] The C3(H20),B complex is formed only in fluid-phase while the C3b,B complex can be formed either in fluid-phase or on a target surface.[1-4] Factor B, which is present in the C3(H20),B or the C3b,B complex, is cleaved into the Ba (33 Kd) and Bb (60 Kd) fragments in the second reaction step by the alternative pathway enzyme, Factor D.[1-4] The resulting C3b,Bb bimolecular complex is the C3 convertase enzyme of the alternative pathway. The Bb subunit is the catalytically active site of the complex that is capable of cleaving C3 to C3a and C3b fragments.[1-4,6] The additional C3b fragments produced in this manner may form the C3b,Bb,C3b trimolecular complex that is the C5 convertase enzyme of the alternative pathway. This C5 convertase is capable of cleaving C5 to C5a and C5b fragments.[1-4,6]

The C3 and C5 convertases of the alternative pathway can be stabilized by Factor P (also called Properdin), a component of the alternative pathway normally present in human plasma or serum,[1-4] or by C3 nephritic factor, an autoantibody produced in some patients experiencing extensive alternative pathway activation.[5] The C3 and C5 convertases of the alternative pathway can be dissociated, and thereby inactivated, by spontaneous decay dissociation,[7] or by the binding of Factor H or Complement Receptor 1 (CR1).[4,8] The Bb fragment that is dissociated from either convertase retains some biological activities, e.g., retention of functional hemolytic activity,[4,9] the ability to induce macrophage- spreading,[10] and plasminogen activation.[11]

Although alternative pathway activation is thought to occur primarily in the absence of specific antibody, many situations arise in which alternative pathway activation can occur as the result of classical pathway activation. For example, immune complexes that are present in autoimmune disease patients can trigger classical complement pathway activation with resultant production of C3b fragments. As described above, these C3b molecules are capable of binding Factor B and initiating its cleavage into the Ba and Bb fragments. Thus, alternative pathway activation can occur in antibody-mediated autoimmune disease states and may contribute significantly to enhanced complement activation and concomitant tissue destruction.

By assessing Factor B cleavage products in test specimens, one can estimate the extent of alternative pathway utilization occurring at the time of sample collection in the disease state under investigation. The MicroVue Bb Plus EIA provides a simple, rapid, non-radioactive, highly specific, and quantitative procedure for measuring Factor B activation. It is ideal for investigations involving the role or status of the alternative complement pathway in numerous research and clinical settings, and for monitoring the generation of Bb *in vitro*.

PRINCIPLE OF THE PROCEDURE

The MicroVue Bb Plus EIA (Enzyme Immunoassay) for the quantitation of Bb in human plasma or serum is a three-step procedure utilizing (1) a microassay plate coated with a mouse monoclonal antibody that binds specifically to human Bb, (2) an HRP-conjugated murine anti-human Bb, and (3) a chromogenic substrate.

In the first step, Standards, Controls, and test specimens are added to microassay wells precoated with a specific anti-Bb monoclonal antibody. Bb, but not Factor B or other complement activation products, present in the Standards, Controls, or specimens will bind to the immobilized anti-Bb monoclonal antibody. After incubation, a wash cycle removes unbound material.

In the second step, horseradish peroxidase (HRP)-conjugated murine anti-Bb antibody is added to each test well. The enzyme conjugated anti-Bb binds to Bb captured in the microassay wells. After incubation, a wash cycle removes unbound, excess conjugate.

In the third step, a chromogenic enzyme substrate is added to each microassay well. The bound HRP-conjugate reacts with the substrate, forming a blue color. After incubation the enzyme reaction is stopped chemically, the color changes to yellow, and the color intensity is measured spectrophotometrically at 450 nm. The color intensity of the reaction mixture is proportional to the concentration of Bb present in the test specimens, Standards, and Controls.

REAGENTS AND MATERIALS PROVIDED

96 Assays for the Bb fragment of Factor B
MicroVue Bb Plus EIA kit contains the following:

A **Bb Plus Standards:** **Parts A9948-A9952** **1 mL each**
B (lyophilized) Each contains a known concentration of Bb in human serum diluted in PBS, protein stabilizers,
C 0.035% ProClin® 300
D
E

L **Bb Plus Low Controls** **Part A9953** **1 mL**
(lyophilized) Contains a known concentration of Bb in human serum diluted in PBS, protein stabilizers, 0.035% ProClin 300

H **Bb Plus High Controls** **Part A9955** **1 mL**
(lyophilized) Contains a known concentration of Bb in human serum diluted in PBS, protein stabilizers, 0.035% ProClin 300

❶ **Microassay Plate** **Part A9559** **12 x 8 wells**
12 eight-well strips coated with a purified mouse monoclonal antibody specific for human Bb in a resealable foil pouch

❷ **Stop Solution** **Part A9947** **12 mL**
Contains 1N Hydrochloric acid

❸ **20X Wash Solution Concentrate** **Part A9957** **50 mL**
Each contains phosphate buffered saline (PBS), 1.0% Tween-20®, and 0.035% ProClin 300

❹ **Complement Specimen Diluent** **Part A3670** **50 mL**
Contains PBS, 0.05% Tween-20, 2.5% protein stabilizers, 0.035% ProClin 300

❺ **TMB Substrate** **Part 5059** **12 mL**
Contains 3,3', 5,5' tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2)

❻ **Bb Plus Conjugate** **Part A9956** **7 mL**
Contains horseradish peroxidase-conjugated murine anti-human Bb suspended in HRP stabilizing buffer with preservative

❽ **Hydrating Reagent** **Part A3675** **25 mL**
Contains 0.035% ProClin 300

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer (60 minute range)
- Calculator or other computational method to validate the assay
- Clean, unused microassay plates and/or test tubes and racks
- Container for wash buffer dilution
- Wash bottle or other immunoassay washing system
- Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes (optional)
- Clean pipettes, 1 mL, 5 mL, and 10 mL
- Micropipettes and pipette tips
- Plate reader capable of optical density readings between 0.0 and 2.0
- Deionized or distilled water

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Use of Heparin Plasma in this assay may give erroneous results.
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
- Store assay reagents as indicated.
- Do not use Coated Strips if pouch is punctured.
- When adding or aspirating liquids from the microassay wells, do not scrape or touch the bottom of the wells.
- Using incubation times and temperatures other than those indicated in the Procedure section may give erroneous results.
- Do not allow microassay wells to dry once the assay has begun.
- Do not use a microassay well for more than one test.
- Use of multichannel pipettes or repeat pipettors is recommended to ensure timely delivery of reagents.
- For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
- Proper collection and storage of test specimens are essential for accurate results (see *SPECIMEN COLLECTION AND PREPARATION*, page 6).
- Avoid microbial or cross-contamination of specimens, reagents, or materials. Incorrect results may be obtained if contaminated.
- Each donor unit used in the preparation of the Standards and Control sera was tested by an FDA-approved method for the presence of antibody to human immunodeficiency virus, (HIV 1 and 2) and hepatitis C virus, as well as for hepatitis B surface antigen and found to be negative (were not repeatedly reactive). However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Micro-biological and Biomedical Laboratories" 1999.

- ProClin 300 is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes, or mouth. Use good laboratory practices to reduce exposure. Seek medical attention if symptoms are experienced.
- **The Substrate is light sensitive. Avoid prolonged exposure to bright or direct light. Store reagents in the dark when not in use.**
- To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
- **A wash bottle should be used to wash the plate. For best results, do not use a multichannel pipette to wash the microassay plate.**
- Heat-inactivated, hyperlipemic or contaminated specimens may give erroneous results.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

REAGENT PREPARATION

Bring all reagents and materials to 15°C to 25°C before use.

After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see *STORAGE*).

Coated Strips

Determine the number of strips needed for the assay. Remove the desired number of strips. Secure the selected strips that are to be used in the plate frame. Place the unneeded strips back into the storage bag, seal the bag, and store at 2°C to 8°C.

Wash Solution

Prepare the Wash Solution for washing the micro-assay wells by diluting 50 mL of the 20X Wash Solution Concentrate up to a final volume of one liter with distilled or deionized water. Mix thoroughly before use. The Wash Solution is stable for 30 days when stored in a clean container at 2°C to 8°C. If cloudiness occurs, discard the reagent.

Bb Plus Standard and Control Reconstitution

Add 1.0 mL of Hydrating Reagent to each Standard vial (A - E), and to the Low Control and the High Control. Allow the reconstituted vials to rehydrate for at least 15 minutes at room temperature. Mix thoroughly. Avoid formation of foam or bubbles during mixing. Reconstituted standards and controls are stable for 30 days when stored at 2°C to 8°C.

Specimen Dilution

Caution: Treat all specimens as if potentially infectious. Do not use heat-inactivated or contaminated specimens.

It is recommended that plasma samples be diluted 1:10 in Specimen Diluent for use in the MicroVue Bb Plus EIA. It is recommended that serum samples be diluted 1:20 in Specimen Diluent. Once diluted, the specimens must be added to the microassay wells within 30 minutes. Do not store or re-use diluted specimens. Any remaining specimens should be discarded.

Specimens with high levels of complement activation may require larger sample dilutions than those indicated.

Adding Diluted Specimens to the Microtiter Wells

Either of two methods can be used to add diluted specimens, Standards, Controls, and Buffer, to the wells (see Step 3 of *ASSAY PROCEDURE*). For small assay runs where only a few specimens are being tested, the diluted specimens and other reagents can be added directly to their assigned wells with a micropipette (100 µL/well). For small or large runs, but especially larger runs, Quidel recommends the use of a multichannel pipettor for adding specimens as follows. (**A multi-channel pipettor may be used to conveniently add the Conjugate, Substrate and Stop Solution, as well**).

In order to load the Standards, Controls and diluted specimens into the microassay wells as rapidly as possible, a "replica plating" procedure can be employed. Instead of adding 100 µL of each Standard, Control, or diluted specimen to the antibody-coated wells individually, 120-130 µL of each solution can be added to individual wells in a blank plate (not provided) corresponding to the final EIA pattern desired. After all the solutions to be tested have been added to the microassay wells in the blank plate, rapidly transfer 100 µL from each blank well to the antibody-coated wells using a multichannel micropipettor. To avoid the possibility of cross-contamination, pipette tips must be changed each time there is a change in the composition of the samples to be transferred.

STORAGE

Store the unopened kit at 2°C to 8°C. After the kit is opened, the 20X Wash Solution Concentrate and Hydrating Reagent may be stored at 2°C to 25°C.

All reagents must be brought to room temperature (15°C to 25°C) before use. Place all unused microassay strips into the storage bag, reseal the bag, and store at 2°C to 8°C.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Cloudiness of the Wash Solution indicates a deterioration of this reagent. If this occurs, the solution should be discarded.

SPECIMEN COLLECTION AND PREPARATION

Handle and dispose of all specimens using Universal Precautions.

The proper collection and storage of specimens is essential, since the Bb fragment of Factor B is susceptible to proteolysis in improperly collected or stored specimens.

Due to complement activation that occurs during clotting, the Bb concentration in normal human serum samples will be higher than those obtained with EDTA plasma samples. The Bb levels in EDTA plasma may therefore more accurately represent the in vivo concentrations.

Serum or EDTA plasma specimens should be collected aseptically using standard techniques. The specimens should be tested immediately or stored at 4°C or on ice until assayed. However, this short-term storage on ice should not exceed four hours.
For longer-term storage, serum or plasma should be frozen at –70°C or below within two hours after collection.

Thaw frozen (≤–70°C) specimens rapidly in a 37°C water bath until just thawed. Transfer thawed specimens immediately to ice (for no longer than four hours) to prevent complement activation prior to dilution. **Do not**

leave specimens at 37°C. Do not thaw specimens at room temperature or 4°C, as this can lead to complement activation. Frozen specimens should be tested as soon as possible after thawing. Repeated freezing and thawing is not recommended. If samples are to be re-frozen for further analysis, we suggest freezing multiple aliquots of the specimen to prevent repeated freeze/thaw cycles.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See *WARNINGS AND PRECAUTIONS* and *REAGENT PREPARATION*.

1. Record the microassay well positions corresponding to the blank well(s), all test samples, Standards, and Controls, as well as the indicated lot numbers from the vial labels. Label one corner of the Microassay Plate for orientation.
2. Prepare the microassay strips as follows:
   a. Using a wash bottle, add approximately 300 µL Wash Solution to each well. **NOTE: For best results, do not use a multichannel pipette to wash the microassay plate.**
   b. Incubate the wells for one minute at 15°C to 25°C.
   c. Aspirate the contents from each well.
   d. Add approximately 300 µL Wash Solution to each well.
   e. Aspirate the contents from each well.
   f. **Repeat steps d-e one more time, for a total of three washes**.
   g. Invert the plate and tap firmly on absorbent paper to remove any remaining liquid.
3. Add 100 µL of Specimen Diluent, reconstituted Standards, Controls, or diluted specimens to the assigned wells.
4. Incubate at 15°C to 25°C for 30 ± 1 minutes.
5. Wash the microassay wells a total of 5 times using the following procedure:
   a. Aspirate the contents from each well.
   b. Using a wash bottle, add approximately 300 µL Wash Solution to each well. **NOTE: For best results, do not use a multichannel pipette to wash the microassay plate.**
   c. Incubate the wells for 1 minute at 15°C to 25°C.
   d. Aspirate the contents from each well.
   e. Add approximately 300 µL Wash Solution to each well.
   f. Aspirate the contents from each well.
   g. Repeat steps e-f three additional times.
   h. After the fifth wash cycle, invert the plate and tap firmly on absorbent paper to remove any remaining liquid.
6. Using a multichannel or repeating pipette, dispense 50 µL of Bb Plus Conjugate into each washed test well, including the blank well(s).
7. Incubate the microassay strips at 15°C to 25°C for 30 ± 1 minutes.
8. Wash the microassay wells after the 30-minute incubation (step 7), as described under *ASSAY PROCEDURE*, step 5. **NOTE: For best results, do not use a multichannel pipette to wash the microassay plate.**
9. Immediately following the wash procedure, dispense 100 µL of the TMB Substrate Solution into each well, including the blank(s). **NOTE: The TMB Substrate must be protected from light during storage and incubation. The TMB Substrate should not be dispensed into a reagent reservoir or other apparatus where light exposure could occur until immediately before dispensing into the microassay wells.**
10. Incubate the microassay strips at 15°C to 25°C for 15 ± 1 minutes in the dark.
11. Add 100 µL of Stop Solution to each well to stop the enzymatic reaction. The Stop Solution should be added to the wells in the same order and at the same rate as the Substrate Solution had been added.
12. Gently tap the plate on the bench top to disperse the color development completely and evenly.
13. Determine the absorbance reading at 450 nm for each test well within one hour after the addition of the Stop Solution (step 11), making a blank correction in accordance with the spectrophotometric system in use.

14. Dispose of the remaining diluted specimens, Controls, substrate, and the used microassay strips (see *WARNINGS AND PRECAUTIONS*).

QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation. The optical density values provided are intended as a guideline only. The results obtained by your laboratory may differ.

Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable, and the samples should be repeated.

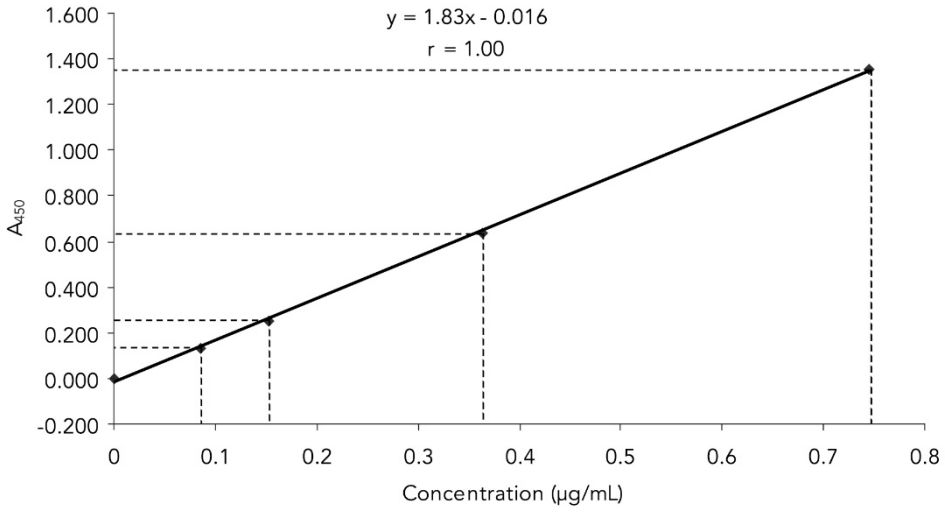
INTERPRETATION OF RESULTS

Use of the Standard Curve

The standard curve for the Bb EIA is generated using the blank subtracted A_{450} values for each Standard (on the y axis) and the assigned concentration for each Bb Plus Standard (on the x axis). After linear regression, the generated standard curve must meet the validation requirements (see below). Most computers and calculators are capable of performing these calculations.

Alternatively, the data may be graphed manually and the values (µg/mL) of the test samples read directly from the best-fit line of the standard curve. An example of a typical standard curve is shown in Figure 1.

Figure 1.
Representative Standard Curve



Calculation of Actual Bb Concentration in Test Specimens

The actual Bb concentration present in each undiluted test specimen is determined by multiplying the Bb/mL concentration, determined from the Kit Standard Curve, by the reciprocal of the specimen dilution factor used.

If the A_{450} values for a given test specimen are greater than that of the highest Standard (E), the results should be reported as "greater than" the Bb concentration of the highest Standard (E) multiplied by the specimen dilution factor. If a more accurate Bb concentration value is required, the test specimen should be re-assayed using a larger dilution factor. In all repeat assays, the Bb Plus Standards and Controls must also be run.

VALIDATION

Determine the slope, intercept, and correlation coefficient of the derived best-fit line. The values must be within the specified ranges to qualify the assay:

correlation coefficient (r):	> 0.96
slope (m):	between 1.094 and 2.558
y-intercept (b):	between (-) 0.145 to 0.113

Refer to the certificate of analysis for the mean acceptable Bb concentration ranges for the High and Low Controls.

LIMITATIONS

The MicroVue Bb Plus EIA has been used to test specimens collected as serum or as plasma in EDTA. Heparin plasma is NOT suitable for this assay. Other anticoagulants have not been tested.

PERFORMANCE OF THE TEST

Limits

LOD: The limit of detection (LOD) for the Bb Plus EIA is 0.018 µg/mL, determined by the upper 3SD limit in a zero standard study.

LLOQ: The lower limit of quantitation (LLOQ) for the Bb Plus EIA is 0.033 µg/mL, the lowest concentration on the standard curve that met NCCLS criteria for accuracy and precision.

ULOQ: The upper limit of quantitation (ULOQ) for the Bb Plus EIA is 0.836 µg/mL, the highest concentration that met NCCLS criteria for accuracy and precision.

Interfering Substances

Na+ Heparin at 14 U/mL (the concentration consistent with Heparin plasma collection tubes) interferes with the Bb Plus EIA and is therefore not recommended for use as a plasma anticoagulant for sample collection. The following substances were tested in the Bb Plus EIA and found to not interfere with the assay:

Substance	Concentration
Bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3000 mg/dL
Albumin	6000 mg/dL
Glucose	1200 mg/dL
Cholesterol	500 mg/dL

Precision

Within-run and between-run precision was determined by assaying 20 replicates of 2 plasma samples and 2 serum samples in 10 different runs.

Sample	Bb (µg/mL)	Within-run[1] C.V. (%)	Between-run[2] C.V. (%)
EDTA Plasma	1.550	2.4	7.7
	0.517	2.5	6.7
Serum	2.129	3.1	6.2
	2.375	4.0	9.1

[1]n = 20 replicates [2]n = 10 runs

Linearity

Linearity was performed by serially diluting samples and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Observed Bb (µg/mL)	Expected Bb (ng/mL)3	Recovery (%)
EDTA Plasma	1:10	0.160	*	*
	1:16	0.107	0.100	106.9
	1:20	0.079	0.080	98.7
	1:32	0.052	0.050	103.9
Serum 1	1:20	0.161	*	*
	1:32	0.103	0.101	102.0
	1:40	0.069	0.081	85.4
	1:64	0.044	0.050	87.2
Serum 2	1:20	0.597	*	*
	1:25	0.467	0.478	97.7
	1:30	0.420	0.398	105.4
	1:40	0.310	0.299	103.8
	1:50	0.230	0.239	96.2
	1:60	0.196	0.199	98.4
	1:80	0.133	0.149	89.0

SAMPLE VALUES

EDTA plasma and serum from thirty-six (36) and forty-nine (49) normal donors, respectively, were tested in the MicroVue Bb Plus EIA kit. The results are presented below.

	n	mean	RANGE ±2 SD	RANGE ±3 SD
EDTA Plasma	36	0.96 µg/mL	0.49-1.42 µg/mL	0.26-1.65 µg/mL
Serum	49	3.53 µg/mL	0.80-6.26 µg/mL	0.0-7.62 µg/mL

Note: The mean and Standard Deviation (SD) behavior of Bb fragment concentrations determined for plasma or serum samples may vary between laboratories. Therefore, it is recommended that each laboratory determine the mean Bb fragment concentration and standard deviation values for samples

ASSISTANCE

To place an order or for technical support, please contact a Quidel representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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GLOSSARY

REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
 Consult e-labeling instructions for use	 Biological risks
IVD For *In Vitro* diagnostic use	 Contains sufficient for 96 determinations
CONT Contents/Contains	CONTROL Control