ANTIBODY TO CYTOMEGALOVIRUS (CMV)
ABBOTT CMV TOTAL AB EIA

NOTE CHANGES HIGHLIGHTED

NAME AND INTENDED USE
ABBOTT CMV TOTAL AB EIA IS A SOLID PHASE ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETECTION OF ANTIBODY TO CYTOMEGALOVIRUS (CMV) IN HUMAN SERUM OR PLASMA AS AN INDICATION OF PAST OR CURRENT INFECTION WITH CMV. THIS PRODUCT IS INTENDED AS A SCREEN FOR THE PRESENCE OF ANTIBODY TO CMV IN BLOOD OR PLASMA DONORS AND SHOULD NOT BE USED AS AN AID IN THE DIAGNOSIS OF CMV INFECTION.

69-4081/R8

WARNING: A software upgrade and/or protocol edits may be required prior to implementing this assay. Please contact your local Customer Support Center.

ABBOTT LABORATORIES
Diagnostics Division

CUSTOMER SUPPORT CENTER (USA)
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Abbott Park, IL 60064
List No. 6163

Printed in U.S.A.

NAME AND INTENDED USE

ABBOTT CMV Total AB EIA is a solid phase enzyme immunoassay for the qualitative detection of antibody to cytomegalovirus (CMV) in human serum or plasma as an indication of past or current infection with CMV. This product is intended as a screen for the presence of antibody to CMV in blood or plasma donors and should not be used as an aid in the diagnosis of CMV infection.

SUMMARY AND EXPLANATION OF THE TEST

Cytomegalovirus (CMV) is one of the leading causes of congenital viral infections[1] and has been associated with infections following blood transfusions,[2,3] renal transplants[4] and bone marrow transplants.[5] Transfusion of blood from seropositive donors to seronegative recipients has been implicated as a source of CMV infection.[6-10] The frequency of seropositivity to CMV in random blood donor populations worldwide has been reported to range from 40 to 90%.[2] Generally, the incidence of post transfusion infection is related to the number of units received rather than to the condition for which transfusion is given.[3,9] A growing number of studies indicate that exclusion of seropositive blood donors may significantly reduce CMV infection in special risk seronegative recipients.[11]

Enzyme immunoassays, first described by Engvall and Perlman[12] and Van Weemen and Schuurs[13] in 1971, are both specific and sensitive for detecting and measuring serum proteins.

A variety of methods have been developed to detect antibodies to CMV including enzyme immunoassay,[14] indirect fluorescent antibody (IFA),[15] indirect hemagglutination (IHA),[16,17] complement fixation (CF),[18,19] and fluorescent immunoassay (FIA).[20]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

In the ABBOTT CMV Total AB EIA, polystyrene beads coated with heat inactivated CMV antigen are incubated with diluted serum, plasma or appropriate controls. Any antibody to CMV that is present is bound to the antigen on the solid phase. After aspiration of the unbound material and washing of the beads, anti-human immunoglobulin (containing antibodies against IgA, IgG, IgM, heavy and light chains) conjugated with horseradish peroxidase (HRPO) is allowed to react with the antigen-antibody complex on the beads. Unbound enzyme conjugate is then aspirated and the beads are washed. Next, o-Phenylenediamine (OPD) Solution containing hydrogen peroxide is added to the beads and, after incubation, a yellow-orange color develops in proportion to the amount of antibody to CMV bound to the beads. The enzyme reaction is stopped by the addition of 1 *N* Sulfuric Acid. The absorbance of Negative Calibrator/Positive Control and specimens is determined using a spectrophotometer with the wavelength set at 492 nm. Specimens giving absorbance values equal to or greater than the Cutoff are considered reactive for antibody to CMV.

REAGENTS

The reagent used to calculate the CMV Total Ab EIA assay results is referred to as the Negative "Calibrator" (NC). Instrument and/or data management product literature and printouts used with the CMV Total Ab EIA assay may refer to NC as Negative "Control and/or ("NCN1")". This difference in terminology does not affect assay results.

No. 6163 ABBOTT CMV Total AB EIA Kit, 100/500 Tests

①	100/500 CMV (AD169) Antigen Coated Beads (Inactivated).
②	1 Vial (27 mL)/1 Vial (115 mL) Enzyme Conjugate CMV Total AB EIA. Antibody to Human Immunoglobulin (Goat): Peroxidase (Horseradish). Minimum Concentration: 20 ng/mL in protein stabilizers with Antimicrobial Agents.
③	1 Vial (6 mL)/3 Vials (6 mL each) Positive Control CMV Total AB EIA. Human Serum in protein stabilizers with Antimicrobial Agents. Reactive for antibody to CMV, nonreactive for HBsAg, anti-HCV, HIV-1 Ag, and anti-HIV-1/HIV-2 by FDA licensed tests.
④	1 Vial (10 mL)/3 Vials (10 mL each) Negative Calibrator CMV Total AB EIA. Human Serum in protein stabilizers with Antimicrobial Agents. Nonreactive for HBsAg, anti-CMV, anti-HCV, HIV-1 Ag, and anti-HIV-1/HIV-2 by FDA licensed tests.
⑤	1 Vial (40 mL)/5 Vials (40 mL each) Specimen Dilution Buffer CMV Total AB EIA. Buffered Calf Serum with Antimicrobial Agents.
*	There are no reagents 6, 7 and 8.
⑨	1 Bottle (20 Tablets)/1 Bottle (40 Tablets) OPD (o-Phenylenediamine • 2 HCl and Sodium Carbonate) Tablets. 12.8 mg OPD/Tablet.
⑩	1 Bottle (110 mL)/1 Bottle (220 mL) Diluent for OPD (o-Phenylenediamine • 2 HCl and Sodium Carbonate) Citrate-Phosphate Buffer containing 0.02% Hydrogen Peroxide.

The stopping reagent is provided as an accessory to the ABBOTT CMV Total AB EIA Kit and consists of:

⑪ 1 *N* Sulfuric Acid, No. 7212 (most U.S. and International locations).

Use of acid other than that supplied by ABBOTT may result in instability of the developed color. To be suitable as a stopping reagent, Sulfuric Acid must pass the following test each time it is prepared. The following test cannot be performed on the COMMANDER® Parallel Processing Center (PPC) (all specific references to procedures for the COMMANDER® System or the PPC alone will be highlighted in green). Use a Quantum™ II, Quantumatic™ or suitable spectrophotometer to perform this test.

1. Pipette 300 µL of OPD Substrate Solution into 5 EIA reaction tubes or acid washed/distilled or deionized water rinsed tubes.
2. Add 1 mL of the 1 *N* Sulfuric Acid under test to each of the five tubes.
3. Measure the A_{492} of the OPD/Acid Solution against distilled or deionized water at "0 TIME" and "120 MIN".
4. Calculate the Mean Absorbance at "0 TIME" and "120 MIN".
5. To be acceptable, acid must exhibit:
   a. an A_{492} of less than 0.040 at "0 TIME" and
   b. a difference of less than 0.030 units in the values obtained at "0 TIME" and "120 MIN".

ADDITIONAL REAGENTS AVAILABLE (Most International Locations):

- 1 *N* Sulfuric Acid, No. 7212-01 (110 mL).
- 6 *N* Sulfuric Acid, No. 7212-03 (110 mL).

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE

This product contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive for HBsAg, HIV-1 Ag, anti-HCV and anti-HIV-1/HIV-2. 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.[21] Biosafety Level 2 [22] or other appropriate biosafety practices [23,24] should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to the following:

1. Wear gloves when handling specimens or reagents.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
4. Clean and disinfect all spills of specimens or reagents using a tuberculocidal disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.[25,26]
5. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state and federal regulations.[27,28]

- Avoid contact of OPD and Sulfuric Acid with skin and mucous membranes. If these reagents come into contact with skin, wash thoroughly with water.
- The OPD tablets listed in the **REAGENTS** section of this package insert contain o-Phenylenediamine • 2 HCl and Sodium Carbonate. The OPD tablets are classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

R40/22	Harmful: possible risks of irreversible effects if swallowed.
R36/38	Irritating to eyes and skin.
R43	May cause sensitization by skin contact.
S2	Keep out of the reach of children.
S13	Keep away from food, drink and animal feedingstuffs.
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
S46	If swallowed, seek medical advice immediately and show this container or label.

Handling Precautions

- Do not use kit beyond the expiration date.
- Do not mix reagents from different lots.
  NOTE: Any OPD Diluent lot, OPD Tablet lot, or 1 *N* Sulfuric Acid lot may be used with any ABBOTT EIA kit.
- Avoid microbial contamination of specimens, reagents and water used for washing. Use of disposable pipette tips is recommended.
- Avoid chemical contamination of reagents and equipment.
- Do not expose OPD reagents to strong light during storage or incubation.
- Avoid contact of the OPD Substrate Solution and 1 *N* Sulfuric Acid with any oxidizing agent. Do not allow OPD Substrate Solution or 1 *N* Sulfuric Acid to come in contact with any metal parts. Prior to use, rinse glassware used with OPD Substrate Solution thoroughly with 1 *N* Acid (sulfuric or hydrochloric) using approximately 10% of the container volume followed by three rinses of distilled or deionized water at the same volume.
- If the desiccant obstructs the flow of beads, remove from bead bottle prior to dispensing beads. Replace desiccant in bottle and tightly cap bottle for storage. Do not store beads with dispenser attached to bottle.

  CAUTION: Do not open the bead bottle until it is at room temperature.
- USE A CLEAN DEDICATED DISPENSER FOR THE CONJUGATE SOLUTION TO AVOID NEUTRALIZATION.
- Use the Negative Calibrator and Positive Control as provided. They

should not be diluted.

- Ensure that the specimen is added to the reaction well. If a specimen is inadvertently not added, the assay may yield an ERRONEOUS, nonreactive result.
- Inadequate adherence to package insert instructions may result in ERRONEOUS results.
- Use accurately calibrated equipment.

INSTRUCTIONS FOR PREPARATION OF OPD SUBSTRATE SOLUTION

Bring OPD Reagents to room temperature (15 to 30°C).

CAUTION: Do not open OPD Tablet bottle until it is at room temperature.

At least five minutes prior to dispensing for color development, prepare the OPD Substrate Solution by dissolving the OPD (o-Phenylenediamine • 2 HCl and Sodium Carbonate) Tablet(s) in Diluent for OPD. DO NOT USE A TABLET THAT IS NOT INTACT.

Using clean measuring devices capable of maintaining a delivery volume tolerance of ± 10% and metal-free containers (such as plasticware or acid-washed and distilled or deionized water-rinsed glassware) follow the procedure below:

1. Transfer into a suitable container 5 mL of Diluent for OPD for each tablet to be dissolved.
2. Transfer appropriate number of OPD Tablets (see OPD Preparation Chart) into measured amount of Diluent for OPD using a nonmetallic forceps or equivalent. Return desiccant to bottle immediately, if removed to obtain a tablet, and close bottle tightly. Allow tablet(s) to dissolve. Do not cap or stopper the Substrate Solution bottle while the tablets are dissolving.

   NOTE: The OPD Substrate Solution **MUST** be dispensed to begin OPD incubation step within 60 minutes of preparation and **MUST NOT** be exposed to strong light. Record the preparation time and expiration time of the OPD Substrate Solution.
3. Just prior to dispensing for Color Development, swirl container gently to obtain a homogeneous solution. Remove air bubbles from dispenser tubing, and prime dispenser prior to use.

OPD PREPARATION CHART

No. Tests	Tablets	Diluent
13	1	5 mL
28	2	10 mL
43	3	15 mL
58	4	20 mL
73	5	25 mL
88	6	30 mL
103	7	35 mL
118	8	40 mL
133	9	45 mL
148	10	50 mL

NOTE: 300 µL of OPD Substrate Solution is required for each specimen or Negative Calibrator/Positive Control as well as for each substrate blank. Laboratories using the COMMANDER® Parallel Processing Center (PPC) will require additional OPD Substrate Solution for instrument priming.

STORAGE INSTRUCTIONS

- Store kit reagents and OPD diluent at 2 to 8°C. OPD Tablets and 1 *N* Sulfuric Acid may be stored at 2 to 30°C. Do not freeze kit reagents.
- Bring all reagents to room temperature (15 to 30°C) for use and return them to storage conditions indicated above immediately after use.

  CAUTION: Do not open the bead bottle or the OPD Tablet bottle until it is at room temperature.
- Retain desiccant in the bead bottle and in the OPD Tablet bottle at all times during storage.
- Reconstituted OPD Substrate Solution (OPD plus Diluent for OPD) **MUST** be stored at room temperature and **MUST** be dispensed to begin OPD incubation step within 60 minutes. Do not expose to strong light.
- Replace desiccant in bead bottle and tightly cap for storage.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

The OPD Substrate Solution should be colorless to pale yellow. A yellow-orange color indicates that the reagent has been contaminated and must be discarded. Precipitates in reagent solutions are generally considered indications of reagent instability or deterioration and must be discarded.

A value of less than 0.600 absorbance units for the difference between the Positive Control and Negative Calibrator Means (P-N) may indicate technique errors or deterioration of the kit reagents or OPD reagents. Such runs must be repeated.

SPECIMEN COLLECTION AND PREPARATION

- Either human serum (including serum collected in serum separator tubes) or plasma collected in ACD, sodium citrate, CPDA-1, CPD, CP2D, sodium EDTA, potassium EDTA, sodium heparin or potassium oxalate based anticoagulant may be used in the ABBOTT CMV Total AB EIA test. The correct ratio of anticoagulant quantity to specimen volume, as recommended by the manufacturer of the anticoagulant, is required. Remove the serum or plasma from the clot or red cells as soon as possible to avoid hemolysis.
- No qualitative performance differences were observed when ABBOTT CMV Total AB EIA nonreactive and reactive samples were tested with elevated levels of bilirubin (≤ 25 mg/dL), hemoglobin (≤ 2000 mg/dL), or lipids (≤ 3000 mg/dL).
- Specimens containing precipitate may give inconsistent test results. Such specimens should be clarified by centrifugation prior to assaying.
- Do not use heat-inactivated specimens.
- Performance has not been established using cadaver specimens or body fluids other than human serum or plasma.
- If specimens are to be stored, they may be stored on or off the clot or red blood cells at 2 to 8°C for up to 14 days. However, if storage periods greater than 14 days are anticipated, the serum or plasma specimens must be removed from the clot or red cells and stored frozen at -10°C or colder. More than three freeze-thaw cycles should be avoided. Specimens must be mixed thoroughly after thawing prior to testing.
- If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents. Specimens may be shipped either ambient, refrigerated (2 to 8°C) on wet ice, or frozen(-10°C or colder) on dry ice.
- Specimens with obvious microbial contamination should not be used.
- All glassware or plastic materials coming into contact with the specimen must be free of any residue from previous specimens, reagents, or cleaning compounds.

PROCEDURE

Materials Provided

No. 6163 ABBOTT CMV Total AB EIA Kit, 100/500 Tests

(See **REAGENTS** for a complete listing)

The list of accessories required for the COMMANDER® Flexible Pipetting Center (FPC) and Parallel Processing Center (PPC) are found in the appropriate COMMANDER® Operations Manual(s). A combination of accessories is included with the COMMANDER® FPC and PPC. ABBOTT CMV Total AB EIA is designed to be compatible with the COMMANDER® FPC and PPC. The product may be used with a Quantum™ II or Quantumatic™.

An optimum combination of the following accessories may be provided:

- Reaction Trays
- Cover Seals
- Assay Tubes with Identifying Cartons
- 1 *N* Sulfuric Acid, No. 7212 (Most U.S. and International Locations)

Materials Required But Not Provided

- Precision pipettes with disposable tips, EIA Pipetting Package (No. 7186), or similar equipment to deliver 10 µL, 200 µL, 300 µL, 400 µL (tolerance is ±5%), and 1 mL (tolerance is ±10%).
- Device for delivery of distilled or deionized water such as Gorman-Rupp™ Dispensing Pump or equivalent to deliver a total volume of 20 to 30 mL of rinse solution.
- QwikWash® or device for washing beads such as Pentawash® or equivalent to deliver 4 to 6 mL per well, with a vacuum source, such as Gast® Vacuum Pump, and a double trap for retaining the aspirate and maintaining minimum vacuum of 21 inches of mercury.
- The ProQuantum™ Bead Washer and Reagent Dispenser may also be used in the performance of this assay.
- COMMANDER® Dynamic Incubator or a water bath capable of maintaining temperature between 38 and 42°C.
- COMMANDER® PPC, Quantum™ II or Quantumatic™.
- Disposable, graduated pipettes or dispenser for measuring Diluent for OPD.
- Metal-free containers for the OPD Substrate Solution, can be plasticware or acid-washed glassware which has been rinsed with distilled or deionized water.
- Protective gloves.
- Disinfectant as described in **WARNINGS AND PRECAUTIONS.**
- *• Nonmetallic forceps.
- *• Bead Dispenser.
- *• Membrane Seal Puncture Tool for acid bottles.
- OPD Tray Covers (for COMMANDER® testing).
- Blanking Beads (for COMMANDER® testing).
- Distilled or deionized water.

* Included in EIA Pipetting Package

ABBOTT CMV TOTAL AB EIA TEST PROCEDURE

Preliminary Comments

Laboratories using the COMMANDER® Flexible Pipetting Center (FPC), Dynamic Incubator (DI) or Parallel Processing Center (PPC) should refer to the appropriate COMMANDER® Operations Manual(s) and note special COMMANDER® instructions below. When using other automated instrumentation to deliver Negative Calibrator/Positive Control and specimens, ensure that the instrumentation is compatible with this assay. Follow the manufacturer's directions to achieve the appropriate volumes and dilutions required within the recommended time limits. Precise timing of enzyme immunoassays is critical.

Follow the exact order of specimen and reagent addition as instructed in the steps of the **ASSAY PROCEDURE**.

- Assay three Negative Calibrators and two Positive Controls with each run of specimens. An assay run is defined as a minimum of three Negative Calibrators, two Positive Controls and one specimen on one 20-well or 60-well tray or a maximum of three Negative Calibrators, two Positive Controls and 495 specimens on 20-well or 60-well trays. Ensure that all reaction trays containing Negative Calibrator/Positive Control and/or specimens are subjected to the same processing and incubation times. This may require maintenance of specific time intervals between processing trays. Once the assay has been started, complete all subsequent steps without interruption.

  CAUTION: Use a separate disposable pipette tip for each specimen and Negative Calibrator/Positive Control in order to avoid cross contamination.
- Prior to beginning the assay procedure, bring all reagents to room temperature (15 to 30°C) and mix gently. Adjust the Dynamic Incubator or water bath to 38 to 42°C.
- Identify the reaction tray wells for each Negative Calibrator/Positive Control and specimen.
- After each step, visually verify the presence of solution and bead in each well.
- The exact order of specimen and reagent additions as described in this test procedure must be followed.

PROCEDURAL NOTES

SAMPLE PIPETTING AND DILUTION

CAUTION: DO NOT PIPETTE SPECIMEN DILUTION BUFFER INTO EITHER THE NEGATIVE CALIBRATOR OR POSITIVE CONTROL WELLS.

A. When using the ABBOTT COMMANDER® Flexible Pipetting Center (FPC) and Assay Update Diskette Version 2.02 or higher, pipette Negative Calibrator/ Positive Control, specimens and specimen diluent for PPC processing or non-PPC processing (Quantum™ II or Quantumatic™) using the appropriate FPC Assay Protocol: CMV TL Ab PPC D0, CMV TL Ab PPC D1, CMV TL Ab QT D0, CMV TL Ab QT D1, CMV TL Ab QNB D0, CMV TL Ab QNB D1.

When using these FPC assay protocols, 10 µL of specimen and 200 µL of specimen diluent are pipetted. Negative Calibrator/Positive Control (200 µL) are pipetted with no specimen diluent added.

NOTE: DO NOT DISPENSE SPECIMEN DILUENT INTO TRAYS PRIOR TO PIPETTING ON THE FPC.

NOTE: When using Assay Update Diskette Version 2.5 or higher the FPC Assay Protocols, CMV TL Ab QNB D0 and CMV TL Ab QNB D1 are not applicable.

B. When using a manual method of sample dilution, follow the steps in the "First Incubation" section of the **ASSAY PROCEDURE.**

ASSAY SELECTION ON THE PPC

1. Insert tray and select the appropriate assay number for CMV Total AB EIA. An operator-edited version may be used if the edited lines are consistent with the assay package insert specifications and are supported by documentation at the time of edit. Follow the instructions on the instrument display board.

   When using an automated pipetting device, such as a COMMANDER® Flexible Pipetting Center, verify that the correct PPC assay protocol has been selected for processing.
2. Verify reagent dispenser assignment:

STATION	REAGENT	DISPENSER VOLUME
2	Conjugate	200 µL
4	OPD Solution	300 µL
5	Acid	300 µL

3. BLANKING (COMMANDER® only)

   NOTE: Use ABBOTT COMMANDER® Reagent Blanking Beads only.

   a. During the conjugate incubation step, prepare a "blanks" tray using a separate tray. Place one blanking bead into each of the five wells, A1 through A5.

   b. At the conclusion of the conjugate incubation step, press the Blank key and insert the "blanks" tray, followed immediately by the first assay tray.

   c. At the conclusion of the OPD incubation step, insert the "blanks" tray prior to the first assay tray of the batch.

GENERAL NOTES

- Do not splash specimen or Conjugate outside of well or high up on well rim as it may not be removed in subsequent washings and may cause test interference.
- Use a dedicated pipette which has not been used for human serum to deliver Enzyme Conjugate.
- Verify that the dispensing equipment delivers specified volume and appropriate dilutions for each procedure.
- When using a Bead Dispenser, remove cap from bead bottle, attach Bead Dispenser and dispense beads into wells.
- Ensure cover seals adhere tightly to all wells.
- When washing beads, follow the directions provided with the washing apparatus to provide a total wash volume of 11 to 18 mL for each bead. Use distilled or deionized water.
- Do not splash liquid while tapping trays.

COMMANDER® DYNAMIC INCUBATOR

When using the COMMANDER® Dynamic Incubator, select the STATIC incubation method and the incubation temperature and time designated in the **ASSAY PROCEDURE** section which follows. Use the STATIC incubation method throughout the assay. Failure to use the Dynamic Incubator in the manner described in the Dynamic Incubator Operations Manual may result in incorrect assay results.

COLOR DEVELOPMENT (QUANTUM™ II OR QUANTUMATIC™)

- When transferring beads from wells to assay tubes, align inverted carton of tubes over their respective wells in the reaction tray. Press the tubes tightly over the wells and invert tray and tubes together so that beads fall into corresponding tubes. Blot excess liquid from top of tube carton.
- Avoid strong light during color development.
- Dispense acid in same tube sequence as OPD Substrate Solution.
- Do not allow acid or OPD Substrate Solution to contact metal.

  NOTE: Specimen Diluent, Conjugate and OPD Substrate dispensers must be rinsed with distilled or deionized water after each use. Refer to all dispenser inserts for cleaning procedure.

READING (QUANTUM™ II OR QUANTUMATIC™)

PHOTOMETRIC METHOD

1. Remove air bubbles prior to reading absorbance.
2. Visually inspect blank tubes and discard those that are contaminated (indicated by a yellow-orange color). If both blanks are contaminated, the entire run must be repeated.
3. Determine the absorbance of the substrate blank. In the Mode 0, blank the instrument with the water tube and read the substrate blank as a sample. (Mode 0 refers to Mode 0 on the Quantum™ II and Assay 0 on the Quantumatic™). Stop the Mode 0 assay. The absorbance value of the substrate blank relative to that of the water tube must be greater than or equal to -0.020 and less than or equal to 0.040 in order for the assay to be valid.
4. If the substrate blank is valid, use it to blank the instrument. Read the Negative Calibrator and Positive Control, then read the specimens. If the substrate blank is not valid, repeat steps 3 and 4 using the alternate substrate blank. If both substrate blanks are invalid, the run must be repeated.

ASSAY PROCEDURE (See **Preliminary Comments** and **PROCEDURAL NOTES**)

Laboratories using the COMMANDER® Flexible Pipetting Center (FPC) and Parallel Processing Center (PPC) should follow procedures in the appropriate COMMANDER® Operations Manual(s). When using other automated instrumentation to deliver Negative Calibrator/Positive Control and specimens, follow the manufacturer's directions to achieve the appropriate volumes required. The following assay procedure should be used with the Quantum™ II, Quantumatic™ and PPC when pipetting manually.

CAUTION: Verify that dispensing equipment delivers specified sample and/or reagent volumes and does not introduce cross contamination.

FIRST INCUBATION

1. Pipette 200 µL of the Negative Calibrator or Positive Control into the appropriate reaction tray wells. (3 Negative Calibrators and 2 Positive Controls).
2. Pipette 10 µL of test specimen into the appropriate well.
3. Add 200 µL of Specimen Dilution Buffer to each well containing a test specimen. Do not add dilution buffer to Negative Calibrator/Positive Control wells.
4. Gently tap trays.
5. Add one bead to each well containing a Calibrator, Control or test specimen.

6. Apply cover seal; tap tray gently.
7. Incubate at 38 to 42°C for 55 to 65 minutes in a Dynamic Incubator or water bath.
8. Remove and discard cover seal. Wash each bead immediately.

SECOND INCUBATION

9. Add 200 µL of Conjugate to each reaction well.
10. Apply new cover seal; tap tray gently.
11. Incubate at 38 to 42°C for 28 to 32 minutes in a Dynamic Incubator or water bath.
12. Remove and discard cover seal. Wash each bead immediately.

COLOR DEVELOPMENT

NOTE: The following procedure should be used with the Quantum™ II or Quantumatic™. For PPC processing refer to the PPC Operations Manual.

13. Immediately transfer beads to assay tubes.
14. Prime OPD dispenser immediately prior to dispensing OPD Substrate Solution.
15. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.
16. Cover and incubate at room temperature (15 to 30°C) for 28 to 32 minutes.
17. Add 1 mL of 1 *N* Sulfuric Acid to each tube. Agitate to mix.

READING

18. Blank the instrument with the appropriate substrate blank at 492 nm.
19. Determine absorbance of Negative Calibrator/Positive Control and specimens within 2 hours of addition of acid.

READING RESULTS

INSTRUMENTS

Performance of the ABBOTT CMV Total AB EIA requires the use of a precision spectrophotometer (i.e., COMMANDER® PPC, Quantum™ II or Quantumatic™). REFER TO THE APPROPRIATE INSTRUMENT MANUAL FOR PROPER OPERATION AND CALIBRATION.

NOTE: If software should need to be installed or reloaded, any edited assay protocols must be recreated.

1. Laboratories using the COMMANDER® Parallel Processing Center (PPC) must use software version 6.00 or above. Laboratories using software versions **6.00, 6.01, 6.10, or 6.11** must create an edited Assay Protocol. Edit PPC Assay Protocol #18 to change the assay name to CMV TOTAL AB EIA SCN and the negative gray zone (%) to 0.0. No other Assay Protocol parameters require edits. Verify that all assay protocol parameters of the edited protocol match the assay protocol printed below.

   When using an automated pipetting device such as a COMMANDER® Flexible Pipetting Center, ensure the edited PPC Assay Protocol number is assigned to the correct pipettor Test Number.

PPC ASSAY PROTOCOL

ASSAY PARAMETERS

Assay Number	(As assigned by Operator)
Assay Name	CMV TOTAL AB EIA SCN
Reminder	
SMC Test Number	75
Limit Table	18
Blanking	OPD
Data Reduction	CUTOFF
Well Status Flags	Y
Multiple Trays	Y
Technician Identification	Y
Master Lot	Y
Sample IDs	Y
Number of Blanks	5
Unknown Replicates	1
Wash 1	1
Dispense Volume 1	200
Liquid 1	CMV CONJ
Bottle Location 1	2
Incubation Temperature 1	40
Min Elapsed Time 1	0
Max Elapsed Time 1	0
Wash 2	0
Dispense Volume 2	0
Liquid 2	
Bottle Location 2	1
Incubation Temperature 2	0
Min Elapsed Time 2	0
Max Elapsed Time 2	0
Wash 3	1
Dispense Volume 3	300
Liquid 3	OPD
Bottle Location 3	4
Incubation Temperature 3	25
Min Elapsed Time 3	0
Max Elapsed Time 3	0
300 µL Read	Y
Acid Quench Volume	300
Acid Name	1 N SULFURIC
Acid Bottle Location	5
Negative Replicates	3
Min. Neg. Cntl. Replicates	2
Negative Location	A1
Neg. Min. Absorbance	0.000
Neg. Max. Absorbance	0.080
Negative Aberrant (%):	50.0
Positive Replicates	2
Min. Pos. Cntl. Replicates	2
Positive Location	A4
Pos. Min. Absorbance	N/A
Pos. Max. Absorbance	2.200
Positive Aberrant (%):	N/A
PC2 Replicates	0
Min. PC2 Replicates	0
Min. Cntl. Diff (P-N):	0.600
Negative Control Factor	1.000
Positive Control Factor	0.000
Cutoff Offset	0.075
Reactive Gray Zone (%):	0.0
Negative Gray Zone (%):	0.0*
Reactive Distinction	A
Flagging Definition	R
Min Sample React Abs	N/A
Data Reduction Option	00

***Laboratories using software versions 6.00, 6.01, 6.10 or 6.11 must create an edited assay protocol. Edit PPC Assay Protocol #18 to change the negative gray zone (%) to 0.0.**

Laboratories using COMMANDER® Parallel Processing Center (PPC) software version **8.00 or greater** should process the ABBOTT CMV Total AB EIA using the Assay Protocol CMV TOTAL AB EIA SCN (#33) as provided in the software without editing. If the CMV TOTAL AB EIA SCN Assay Protocol is not available, contact your Abbott Representative for access to the assay protocol.

When pipetting with FPC version 2.5 or higher, the Assay List Number and Assay Procedure Code must match that contained in the PPC Assay Protocol CMV TOTAL AB EIA SCN (#33). When configuring assay protocols in the FPC, ensure the assay procedure code is specified as "SC".

2. Laboratories using the Quantum™ II should read this assay as follows: Laboratories using a Quantum™ II, Module A, List Number greater than 4045-97 should process the ABBOTT CMV Total AB EIA using the assay protocol as provided in the software without editing.

   Laboratories using the Quantum™ II, Module A, List Number 4045-96 or 97 must create an edited assay protocol. Edit mode 1.13 to change the assay name to CMV Total AB SCN and the negative gray zone to 0.0. No other assay protocol parameters require edits. Verify that the edited protocol values and assay name match the protocol values below.

QUANTUM™ II PROTOCOL

Name:	**CMV Total AB SCN**
Filters	=492.600
Path Length	=1.11
Negative Controls	
Replication	=3
Maximum Δ A	=0.080
Aberrant Value Option	=1
Aberrant Cutoff	=50.00
Positive Controls	
Replication	=2
Aberrant Value Option	=0
PC - NC	
Minimum Value	=0.600
Unknowns	
Replication	=1
Cutoff = A*NC + B*PC + C	
A	=1.000
B	=0.000
C	=0.075
Reactive Gray Zone	=0.0
Negative Gray Zone	=0.0*
Distinction	=0
Flag	=0

***Laboratories using the Quantum™ II, Module A, List Number 4045-96 or 97 must create an edited assay protocol. Edit mode 1.13 to change the negative gray zone to 0.0.**

3. Laboratories using the Quantumatic™ should read this assay as follows: Laboratories using a Quantumatic™, List Number greater than 7523-42, should process ABBOTT CMV Total AB EIA using the assay protocol as provided in the software without editing.

   Laboratories using the Quantumatic™, List Number 7523-42, must create an edited assay protocol. Edit the CMV Total AB assay protocol

to change the assay name to CMV Total AB SCN and the negative gray zone to 0.0%. Verify that the edited protocol values and assay name match the protocol values below.

QUANTUMATIC™ PROTOCOL

Name: CMV Total AB SCN
Cutoff Value = (1.000) NCX + (0.000) PCX + 0.075
P>N

No. Neg Controls	=3
Max Neg control	=0.080
Neg Aberrant cutoff %	=50.0%
No. Pos Controls	=2
Min Control Diff	=0.600
No. Patient Repl	=1
Reactive Gray Zone	=0.0%
Negative Gray Zone	=0.0%*
No of Pos-2 Controls	=0

***Laboratories using the Quantumatic™, List Number 7523-42, must create an edited assay protocol. Edit the CMV Total AB EIA assay protocol to change the negative gray zone to 0.0%.**

QUALITY CONTROL PROCEDURES

1. Substrate Blank Acceptance Criteria
   a. Quantum™ II/Quantumatic™ users: An assay run is considered valid with respect to the substrate blank if the blank has an absorbance value that is greater than or equal to -0.020 and less than or equal to 0.040. The determination of assay validity due to substrate blank must be done by the user. The substrate blank value is an indication of the integrity of the OPD Substrate Solution. If the substrate blank absorbance falls outside the acceptable range, the preparation of the substrate is in question and the alternate substrate blank may be used. If the alternate substrate blank is unacceptable, the assay is invalid, and the run must be repeated.
   b. COMMANDER® PPC users: Quality control with respect to the Substrate Blank is determined automatically by the COMMANDER® PPC according to the procedure described in the PPC Operations Manual. If the run is invalid, technique errors in preparation of the OPD Substrate Solution are suspect and the run must be repeated.

   NOTE: When a Quantum™ II, Quantumatic™, or Parallel Processing Center is used, refer to the appropriate Operations Manual for information concerning calculations performed by the instrument. If one of these instruments is not used, perform the following calculations on the assay data.

2. Negative Calibrator Calculations and Acceptance Criteria
   a. Calculation of Negative Calibrator Mean Absorbance ($NC\bar{x}$).
   Determine the Mean of the Negative Calibrator Values.
   Example:

Negative Calibrator	
Sample No	Absorbance
1	0.028
2	0.030
3	0.032
Total	0.090

$$(NC\bar{x}) = \frac{\text{Total Absorbance}}{3} = \frac{0.090}{3} = 0.030$$

   b. Negative Calibrator Acceptance Criteria
   Individual Negative Calibrator Values should be less than or equal to 0.080. Negative Calibrator Values should also be greater than or equal to 0.5 times $NC\bar{x}$ and less than or equal to 1.5 times $NC\bar{x}$.
   If one value is outside the acceptable range, discard this value and recalculate the mean. If two values are outside the range, the test should be repeated.
   Example:
   0.5 x 0.030 = 0.015
   and
   1.5 x 0.030 = 0.045 Range: 0.015 to 0.045
   In the example, no Negative Calibrator sample is rejected as aberrant and the $NC\bar{x}$ need not be revised.
3. Positive Control Calculations
   Calculation of Positive Control Mean Absorbance ($PC\bar{x}$).
   Determine the Mean of the Positive Control Values.
   Example:

Positive Control	
Sample No.	Absorbance
4	0.900
5	1.200
Total	2.100

$$(PC\bar{x}) = \frac{\text{Total Absorbance}}{2} = \frac{2.100}{2} = 1.050$$

4. Assay Run Validity Criteria
   For the run to be valid, the difference between the mean absorbance of the Positive Control and Negative Calibrator (P-N) must be greater than or equal to 0.600. If not, technique or deterioration of reagents may be suspect and the run must be repeated.
   Calculations for Determining P-N:
   Example:
   $PC\bar{x}$ = 1.050
   $NC\bar{x}$ = 0.030
   P-N Value = (1.050 - 0.030) = 1.020

RESULTS

When a COMMANDER® PPC, Quantum™ II or Quantumatic™ is used, all calculations below are performed automatically.

1. Calculation of the Cutoff Value
   The Cutoff Value is the mean absorbance of the Negative Calibrator plus 0.075.
   Example:
   $NC\bar{x}$ = 0.030
   Cutoff Value = 0.075 + $NC\bar{x}$
   = 0.075 + 0.030 = 0.105
2. Calculation of the Unknown
   The presence or absence of antibody to CMV is determined by relating the absorbance of the unknown specimen to the Cutoff Value. If the absorbance of the unknown specimen is greater than or equal to the Cutoff Value, it is considered reactive by the criteria of the ABBOTT CMV Total AB EIA.

INTERPRETATION OF RESULTS

Test specimens with absorbance values greater than or equal to the Cutoff Value are reactive by the ABBOTT CMV Total AB EIA and may be considered positive for antibody to CMV. Test specimens with absorbance values less than the Cutoff Value are nonreactive and may be considered negative for antibody to CMV.

LIMITATIONS OF THE PROCEDURE

- The ABBOTT CMV Total AB EIA may be used to screen for the presence of antibody to CMV in human serum or plasma specimens.
- The ABBOTT CMV Total AB EIA should not be used as an aid in the diagnosis of CMV infection.
- The **PROCEDURE** and **INTERPRETATION OF RESULTS** sections in the ABBOTT CMV Total AB EIA package insert must be followed exactly when testing human serum or plasma specimens for the presence of antibodies to CMV.
- This assay was designed and validated for use with human serum or plasma from individual patient and donor specimens. Pooled specimens must not be used as the accuracy of their test results has not been validated.
- Performance has not been established using cadaver specimens or body fluids other than human serum or plasma.
- Do not use heat-inactivated specimens.
- A test result that is nonreactive does not exclude the possibility of exposure to or infection with CMV. Nonreactive results in this assay in individuals with prior exposure to CMV may be due to antibody levels below the limit of detection of this assay or lack of antibody reactivity to the CMV antigens used in this assay.
- Failure to add specimen in the **PROCEDURE** could result in a false nonreactive test result. Repeat testing should be considered where there is clinical suspicion of CMV infection.

EXPECTED VALUES

The incidence of antibodies to CMV in blood donors throughout the United States has been reported to be as low as 14.8% and as high as 82%.[1,8] The incidence of seropositivity increases with age and is higher in lower socioeconomic groups.[1,9,10]

The performance characteristics of ABBOTT CMV Total AB EIA were evaluated in two separate studies that included specimens from a total of 8312 random blood donors. The incidence of CMV seropositivity as determined by the ABBOTT CMV Total AB EIA was 51.07% in Study 1 and 50.10% in Study 2.

SPECIFIC PERFORMANCE CHARACTERISTICS

ASSAY REPRODUCIBILITY

Assay reproducibility was determined by assaying an eight member panel consisting of specimens from five individuals. Six panel members were matched serum and plasma specimens from each of three CMV antibody nonreactive individuals. The matched plasma and serum specimens from two of the three individuals were spiked with CMV antibody positive serum to borderline and reactive values. Two panel members were sourced from two CMV antibody positive individuals. Using three masterlots, multiple technicians at three sites tested the panel in replicates of three over three consecutive days. The intra- and inter-assay standard deviation (SD) and percent coefficient of variation (%CV) were analyzed with a variance components analysis, using a nested analysis of variance model[29] (Table I).

Mean S/CO is defined as the mean sample absorbance divided by the calculated Cutoff Value.

TABLE I
ABBOTT CMV TOTAL AB EIA Reproducibility

Panel Member	Number of Replicates	Mean S/CO	Intra-assay SD	Intra-assay %CV	Inter-assay* SD	Inter-assay* %CV
1	135	0.249	0.0464	18.7	0.0506	20.4
2	135	0.261	0.0476	18.2	0.0512	19.6
3	135	2.358	0.1772	7.5	0.2915	12.4
4	135	2.000	0.1733	8.7	0.2386	11.9
5	135	10.065	0.5512	5.5	1.0919	10.8
6	135	10.049	0.5905	5.9	1.1286	11.2
7	135	4.067	0.3526	8.7	0.5018	12.3
8	135	4.814	0.3636	7.6	0.5986	12.4

Controls	Number of Replicates	Mean Absorbance	Intra-assay SD	Intra-assay %CV	Inter-assay* SD	Inter-assay* %CV
Negative	135	0.043	0.0068	15.9	0.0079	18.3
Positive	90	1.303	0.0621	4.8	0.1194	9.2

* Inter-assay variability includes intra-assay variability

RELATIVE SPECIFICITY AND DETECTABILITY

Five blood centers evaluated a total of 2312 random blood donor specimens by ABBOTT CMV Total AB EIA and IHA in Study 1 (Table II). Twenty-three specimens were uninterpretable with IHA. Of the remaining 2289 specimens, 51.07% (1169/2289) were positive for antibody to CMV as determined with the ABBOTT CMV Total AB EIA and 49.37% (1130/2289) were positive for antibody to CMV as determined by IHA. The overall agreement between ABBOTT CMV Total AB EIA and IHA was 95.50% (2186/2289). In this study, 506 of the random blood donor specimens were also evaluated by ABBOTT CMV Total AB EIA and IFA (Table III). The overall agreement between ABBOTT CMV Total AB EIA and IFA was 94.07% (476/506).

Of the 2289 specimens tested, 1098 were initially concordant ABBOTT CMV Total AB EIA and IHA positive and 1088 specimens were initially concordant ABBOTT CMV Total AB EIA and IHA negative (Table II). Following retest at the site, 42 of the 103 initially discordant specimens remained discordant.

Twelve of the 42 final discordants were ABBOTT CMV Total AB EIA negative, IHA positive. Eight of these 12 specimens were negative in at least three of the five tests performed at Abbott Laboratories and had a final specimen interpretation of negative. Four of these 12 specimens were not tested further and remained discordant. Relative specificity was calculated to be 99.91% [(1167 - 1)/1167] with a 95% confidence interval of 99.52 to 100.00%.

Thirty of the 42 final discordants were ABBOTT CMV Total AB EIA positive, IHA negative. Four of these 30 specimens were positive in at least three of the five tests performed at Abbott Laboratories and had a final specimen interpretation of positive. Twenty-five of these 30 specimens were negative in at least three of the five tests performed at Abbott Laboratories and had a final specimen interpretation of negative. One specimen was not tested further and remained discordant. Relative Sensitivity was calculated to be 99.64% [(1122 - 4)/1122] with a 95% confidence interval of 99.09 to 99.90%.

TABLE II
STUDY 1
COMPARISON OF ABBOTT CMV TOTAL AB EIA AND INDIRECT HEMAGGLUTINATION ASSAY (IHA)
Initial Results

IHA RESULTS	NUMBER OF SPECIMENS	CMV TOTAL AB EIA POSITIVE	CMV TOTAL AB EIA NEGATIVE
POSITIVE	1130	1098	32
NEGATIVE	1159	71	1088

Results After Retest of Discordants

IHA RESULTS	NUMBER OF SPECIMENS	CMV TOTAL AB EIA POSITIVE	CMV TOTAL AB EIA NEGATIVE
POSITIVE	1126	1114	12
NEGATIVE	1163	30	1133

Results After Resolution of Discordants

FINAL SPECIMEN INTERPRETATION	NUMBER OF SPECIMENS	CMV TOTAL AB EIA POSITIVE	CMV TOTAL AB EIA NEGATIVE
POSITIVE	1122	1118	4
NEGATIVE	1167	1	1166

A subset of 506 serum specimens from random blood donors was also evaluated in Study 1 with an IFA (Table III). Four hundred seventy-six of the 506 (94.07%) specimens had concordant results. The thirty initially discordant specimens were retested with both assays.

Six of the 30 specimens were initially ABBOTT CMV Total AB EIA negative, IFA positive. Five of these six specimens had concordant negative results following retest. The remaining specimen had concordant positive results following retest.

Twenty-four of the 30 specimens were initially ABBOTT CMV Total AB EIA positive, IFA negative. Fifteen of the 24 specimens had concordant positive results following retest. Three of the 24 specimens had concordant negative results following retest. The remaining six specimens continued to have discordant results following retest. These six specimens were also negative with IHA.

TABLE III
STUDY 1
COMPARISON OF ABBOTT CMV TOTAL AB EIA AND INDIRECT FLUORESCENT ANTIBODY (IFA)
Initial Results

IFA RESULTS	NUMBER OF SPECIMENS	CMV TOTAL AB EIA POSITIVE	CMV TOTAL AB EIA NEGATIVE
POSITIVE	247	241	6
NEGATIVE	259	24	235

Results After Retest of Discordants

IFA RESULTS	NUMBER OF SPECIMENS	CMV TOTAL AB EIA POSITIVE	CMV TOTAL AB EIA NEGATIVE
POSITIVE	257	257	0
NEGATIVE	249	6	243

Three blood centers evaluated a total of 6000 random blood donor specimens by ABBOTT CMV Total AB EIA and an agglutination assay in Study 2 (Table IV). Of these specimens, 50.10% (3006/6000) were positive for antibody to CMV as determined with the ABBOTT CMV Total AB EIA and 53.00% (3180/6000) were positive for antibody to CMV as determined by agglutination. The overall agreement between ABBOTT CMV Total AB EIA and agglutination was 95.93% (5756/6000).

Of the 6000 specimens, 2820 were negative by agglutination, 2785 (98.76%) were also negative by ABBOTT CMV Total AB EIA. Twenty-three of the 35 discordant specimens were positive with additional supplemental tests. Relative specificity was calculated to be 99.60% [(2995 - 12)/2995] with a 95% confidence interval of 99.30 to 99.79%.

Of the 3180 specimens positive by agglutination, 2971 (93.43%) were also positive by ABBOTT CMV Total AB EIA. One hundred and ninety-eight of the 209 specimens were negative with additional supplemental tests. Relative Sensitivity was calculated to be 99.63% [(3005 - 11)/3005] with a 95% confidence interval of 99.35 to 99.82%.

TABLE IV
STUDY 2
COMPARISON OF ABBOTT CMV TOTAL AB EIA AND AN AGGLUTINATION ASSAY
Initial Results

AGGLUTINATION RESULTS	NUMBER OF SPECIMENS	CMV TOTAL AB EIA POSITIVE	CMV TOTAL AB EIA NEGATIVE
POSITIVE	3180	2971	209
NEGATIVE	2820	35	2785

Results After Resolution of Discordants

FINAL SPECIMEN INTERPRETATION	NUMBER OF SPECIMENS	CMV TOTAL AB EIA POSITIVE	CMV TOTAL AB EIA NEGATIVE
POSITIVE	3005	2994	11
NEGATIVE	2995	12	2983

A total of 27 CMV antibody negative sera, positive for antibodies to one or more members of the family Herpesviridae, were tested: Epstein-Barr, Herpes Simplex and Varicella-Zoster. All 27 sera were negative with the ABBOTT CMV Total AB EIA. A total of 24 CMV antibody negative sera containing rheumatoid factor was tested with the ABBOTT CMV Total AB EIA. Twenty-two specimens were negative with the ABBOTT CMV Total AB EIA. A total of 11 serum specimens from CMV infected pediatric patients was tested and all specimens were positive for anti-CMV with the ABBOTT CMV Total AB EIA.

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