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BECKMAN COULTER PK® CMV-PA SYSTEM

Passive Particle Agglutination Test for Detection
of Total Cytomegalovirus Antibodies using the PK® Instrument

I. INTENDED USE

The BECKMAN COULTER PK® CMV-PA SYSTEM is a passive particle agglutination assay intended for the qualitative detection of IgG and IgM antibodies to cytomegalovirus (CMV) in human plasma and serum from blood donors using the BECKMAN COULTER PK7200 and/or PK7300 Automated Microplate Systems. A positive result provides evidence of past or current infection with CMV. This test is not intended for diagnostic use.

II. SUMMARY OF TEST

Cytomegalovirus (CMV) is a double-stranded DNA virus with physicochemical characteristics common to members of the herpesvirus family.[1] Serologic surveys have shown that CMV infection is worldwide in distribution, with antibody prevalence in adults in the range of 20-82%.[2-4,18] The majority of CMV infections are subclinical or associated with nonspecific illness. The virus may remain in a latent state indefinitely following initial infection, or it may emerge from time to time to cause an active infection.[5] Pregnant women can transmit the CMV virus to the fetus, resulting in congenital liver, spleen, and/or CNS disease in the unborn child.[6] The congenital effects of mother-to-fetus CMV transmission may be more severe in those cases where the mother has acquired the primary infection during early pregnancy than in maternal cases of reactivated disease.[7] CMV infection often induces life-threatening conditions such as pneumonia, fever, and hepatitis among immunosuppressed patients such as organ transplant recipients and in patients harboring human immunodeficiency virus (HIV).[8,9]

The transfusion or transplant of CMV-seropositive blood or organs may cause a variety of clinical abnormalities in immunocompromised recipients. Since CMV infections are frequently transmitted through organ transplants and blood transfusions,[2-4,10-12] the screening of blood donors for CMV antibodies is an important step toward reducing cytomegalovirus infection in immunocompromised transfusion and transplant recipients.

A variety of methods have been developed to detect antibodies to CMV including indirect hemagglutination assay (IHA), indirect fluorescent assay (IFA), anticomplement immunofluorescence (ACIF), enzyme immunoassay (EIA), or passive latex agglutination (PLA).

In 1951 Boyden[13] succeeded in attaching a variety of protein antigens to the surfaces of tannic acid-treated sheep erythrocytes and demonstrated hemagglutination in the presence of the corresponding antibodies. Variations of these methods are still widely used today despite problems associated with biological carriers. To address these problems, artificial carriers have been developed as substitutes for erythrocytes and are now being used in immune agglutination assays for the detection of antibodies to various infectious diseases.[14-16]

Automation has enhanced the value of the indirect particle agglutination test by significantly reducing the amount of time and labor needed to perform the assay. The BECKMAN COULTER PK CMV-PA SYSTEM has been developed to provide an indirect particle agglutination CMV assay using uniform reagents which are stable, easy to handle, and suitable for use on the BECKMAN COULTER PK7200 and/or PK7300 Automated Microplate Systems.

III. PRINCIPLE OF PROCEDURE

The PK CMV-PA SYSTEM uses gelatin particles coated with cytomegalovirus antigens to detect IgG and IgM antibodies to CMV in human serum and plasma. The test sample or control material is diluted with SAMPLE DILUENT and

then mixed with the sensitized particles in a patented, terraced microplate well. During the incubation, the particles settle in the terraced microplate well. Antibody to CMV will bind to the antigen-sensitized particles during this incubation. Particles with bound antibody will form agglutination, which are visible as a homogeneous blue layer of gelatin particles. When antibodies to CMV are not present, sensitization and subsequent agglutination does not occur. Particles without bound antibody fall freely to the center of the well and visually appear as a compact dense blue button surrounded by a clear zone.

The PK7200 and/or PK7300 instrument will read the settling patterns of particles in each well based on the threshold settings chosen for the reagent. The PK7200 and/or PK7300 determines the presence or absence of antibodies to CMV using a CCD (charged coupled device) camera, which captures the well image allowing differentiation of agglutinated and unagglutinated patterns.

IV.REAGENTS

The PK CMV-PA SYSTEM is available in a kit sufficient to perform 2300 tests. Store reagents at 2-8°C. **DO NOT FREEZE.**

SENSITIZED PARTICLES - 10 vials containing gelatin particles colored with blue dye, sensitized with cytomegalovirus antigens and then lyophilized. Each vial must be reconstituted with 6.0 mL RECONSTITUTING SOLUTION. Reconstituted particles contain 0.15% sodium azide. Each vial is sufficient for 230 tests on the PK7200 and 250 tests on the PK7300.

RECONSTITUTING SOLUTION -1 bottle, 70 mL. Phosphate buffered saline containing 0.10% sodium azide. For the reconstitution of SENSITIZED PARTICLES.

SAMPLE DILUENT - 3 bottles, 300 mL each. Proprietary solution containing phosphate buffered saline, normal rabbit serum and 0.10% sodium azide.

V. WARNING AND PRECAUTIONS

PK CMV-PA SYSTEM is for in vitro diagnostic use.

1) Avoid contamination of reagents or specimens with saliva which can cause indistinguishable agglutination patterns. Do not mouth pipette any reagents.

2) The microplates must be clean and in good condition before use. Damaged plate terraces or improper washing of the microplates resulting in protein buildup or debris in the terraces can adversely affect test results. For instance, the homogenous layer of agglutinated particles in a positive reaction can be disrupted and fold over onto itself. An analogy would be the folding over of the sides of an omelet. This phenomenon can result from excessive vibration, protein buildup in the terraces of the microplate or physical damage to the microplate terraces, and is readily apparent during plate review. The recommended microplate maintenance procedures can be found in the Standard Operating Procedures Manuals for the Beckman Coulter PK7200 Automated *Microplate System and the PK7300 User's* Guide.

3) If positive control samples repeatedly test negative, excessive instrument vibration is a potential cause. When control material repeatedly fails to perform as expected, contact Beckman Coulter Immunohematology Technical Services at 800-447-5852.

4) Avoid freezing of PK CMV-PA reagents and reconstituted SENSITIZED PARTICLES.

5) Sodium azide is added to the reagents as a bacteriostatic agent. Sodium azide has been reported to form explosive lead and copper azides in laboratory plumbing. To prevent azide build-up, flush with large volumes of water if solutions containing azide are disposed of in the sink.

6) Visible signs of microbial growth or gross turbidity in the reagent may indicate

degradation and warrant discontinuance of use.

7) Handle all specimens, human-based reagents and controls as if potentially infectious. Refer to the Center for Disease Control guidelines for handling biological materials.[17]

8) Do not eat, drink or smoke in areas where specimens, human-based reagents and controls are handled.

9) Clean pipettes should be used to reconstitute all reagents. Clean glass or plastic containers should be used for pooling reagents from the same lot.

10) Positive and negative control materials should be handled in the same fashion as donor samples.

11) Inadequate adherence to the package insert can result in erroneous results.

12) Carryover between samples has been detected in some donor samples with high titers of CMV antibodies. The addition of Cleaning Solution to Tank 2 on the Beckman Coulter PK7200 has been found to correct carryover in most cases. Refer to the PK7200 Operator's Manual Section 10. 3. 4. A. for the preparation of the Cleaning Solution. The PK7300 requires the use of Cleaning Solution (no preparation required) in the Detergent Tank, which eliminates carry-over in most cases.

VI. REAGENT PREPARATION

1) Reconstitute, as needed, each vial of SENSITIZED PARTICLES with 6 mL of RECONSTITUTING SOLUTION. Replace the stopper and invert a few times to assure thorough mixing. Prior to use, allow the reagent to reconstitute for a minimum of 30 minutes at room temperature (15-30°C).

2) Reconstituted particles are stable for 7 days at 2-8°C.

3) The date of reconstitution and the reconstituted expiry should be recorded on the reagent vials.

4) After the reconstitution period, gently swirl (DO NOT VORTEX) the reagent to assure thorough resuspension. Follow the steps under heading, "Section X., DIRECTIONS FOR USE", for use on the instrument.

5) SENSITIZED PARTICLES from the same lot number may be pooled following completion of the reconstitution period. The mixture is stable for 7 days from the earliest reconstitution date of the particles contained in the mixture.

6) SENSITIZED PARTICLES from one lot number should not be mixed with those of another lot number.

7) The SAMPLE DILUENT AND RECONSTITUTING SOLUTION are not matrixed to the SENSITIZED PARTICLES lot.

Note: All reagents should be brought to room temperature (15-30°C) before reconstitution and use.

VII. STORAGE

Note: Reagents should not be used after the expiration date.

1) Unused particle suspension may be returned to the original vial, using aseptic technique.

2) Store the PK CMV-PA SYSTEM at 2-8°C. DO NOT FREEZE.

3) The PK CMV-PA SYSTEM should not be used after the expiration date which is printed on the outside of the package.

4) Store reconstituted SENSITIZED PARTICLES at 2-8°C. DO NOT FREEZE. SENSITIZED PARTICLES are stable for 7 days after reconstitution, when stored at 2-8°C.

5) Visible signs of microbial growth or gross turbidity in the reagents may indicate degradation and warrant discontinuance of use.

VIII. SPECIMEN COLLECTION AND PREPARATION

Plasma (EDTA) and serum samples, obtained through standard collection procedures are suitable for this assay. The performance of this assay has not been established with plasma samples employing heparin as the anticoagulant, serum samples collected with serum separator tubes, heat-treated samples, or neonatal samples. In addition, the performance of this assay has not been established with cadaveric samples, pleural fluid, saliva, or nonhuman samples.

Prior to analysis on the PK7200 and/or PK7300, samples should be adequately centrifuged to ensure that the plasma or serum is free from particulate matter. If erythrocytes or other visible components are contained in the sample, remove by centrifugation to prevent interference with the test results. The PK7200 Standard Operating Procedures and/or the PK7300 User's Guide requires centrifugation of samples within 10 hours of analysis and centrifugation for a minimum of 10 minutes at 1000 x g. These requirements exist for the purpose of optimizing red cell sampling. Therefore, plasma or serum samples tested do not need to comply with these requirements as long as the plasma or serum is free from particulate material. Samples exhibiting gross lipemia, hemolysis or icterus may be compromised and may require alternative testing.

EDTA specimens may be tested up to 3 days after collection on the PK7200 and up to 5 days after collection on the PK7300. Sample integrity is best maintained when stored at 2-8°C. However, plasma and serum may be kept at room temperature (15-30°C) for up to 3 days after collection. Serum specimens may be tested up to 14 days after collection when stored at 2-8°C. Serum samples may be stored frozen at<-20°C if testing is to exceed 14 days after collection. Samples should be well mixed after thawing. Repeated freeze/thaw cycles should be avoided. Improper storage of specimens may result in variable settling patterns yielding false positive or indeterminate results.

When shipping specimens, they should be packaged in compliance with applicable federal, state and local regulations covering the transport of clinical specimens and etiologic agents. Specimens may be shipped at either ambient, refrigerated (2-8°C) on wet ice, or frozen (-10°C or colder) on dry ice.

IX. MATERIALS-

MATERIALS PROVIDED IN THE BECKMAN COULTER PK CMV-PA SYSTEM:

-RECONSTITUTING SOLUTION
-SENSITIZED PARTICLES
-SAMPLE DILUENT

MATERIALS REQUIRED BUT NOT PROVIDED:

-Beckman Coulter microplates with a 5µm well terraces
-Pipetting device capable of delivering 6.0 mL
-BECKMAN COULTER PK7200 and/or BECKMAN COULTER PK7300
-BECKMAN COULTER PK® CMV-PA SYSTEM CONTROLS

X. DIRECTIONS FOR USE

The PK Instruments are programmable instruments whose operation is controlled by software. Parameters validated by the manufacturer are incorporated into the operating files. The user may define panel (test) configurations. Please consult Section 8 of the PK7200 Operator's Manual or Section D of the PK7300 User's Guide.

Beckman Coulter has established test parameters and recommended thresholds for the PK7200 and the PK7300 based upon application development with characterized samples. Working files for the PK CMV-PA test are shown below for the PK7200 and PK7300. Good laboratory practice dictates that each laboratory validate the operating parameters.

All reagents, diluents, and specimens should be at room temperature (15-30°C) prior to analysis.

Threshold Settings for the PK7200 and PK7300								
	P/C		SPC		LIA			
	(+) Limit	(-) Limit	Low	High	(+) Limit	(-) Limit	LIA Selection	BG/C
PK7200	24	18	12	14	160	80	5	Low
PK7300	35	20	14	14	200	90	5	Middle

Dynamic Range Settings for the PK7300					
P		C		LIA	
Low	High	Low	High	Low	High
50	99	10	99	0	450

Note: Dynamic Ranges are not defined for the PK7200

PK7200 PARAMETERS

	VOLUME/SETTING	STROKE PIN
Sample Volume	30 µL	
Diluent Volume	250 µL	G 0.25
Ratio	120/1000	
Diluted Sample Volume	25 µL	
Reagent Volume	25 µL	
Reagent Name	CMV	
Channel Designation	(1-12)	
Decision Logic	+/-	
Temperature Setting	28°C	
Incubation Time	60 minutes	
Plate Well	5 µm	

NOTE: REMEMBER TO SAVE SETTINGS ON BOTH THE PROGRAM DISK AND/OR THE HARD DRIVE.

PK7300 PARAMETERS

	VOLUME/SETTING
Sample/Diluent Ratio	8.9
Diluted Sample Volume	25 µL
Reagent Volume	23 µL
Reagent Name	CMV
Channel Designation	(1-12)
Decision Logic	+/-
Temperature Setting	28°C
Incubation Time	60 minutes
Plate Well	5 µm

NOTE: CHANGES MADE TO THE PK7300 ARE AUTOMATICALLY SAVED TO THE HARD DRIVE WHEN THEY ARE MADE. IT IS RECOMMENDED THAT WHEN ANY CHANGES ARE MADE, THEY ALSO BE SAVED TO AN EXTERNAL STORAGE MEDIA.

A. PK7200 PREP Procedure for the BECKMAN COULTER PK CMV-PA SYSTEM :

To use the reagents on the analyzer:

1) Place the reconstituted SENSITIZED PARTICLES into the designated channel of the reagent container. Thorough, uniform mixing of SENSITIZED PARTICLES is important. Prior to placing it on the analyzer, check the reagent container to ensure that the particles are well mixed and have not settled out of suspension. If settling of the particles is observed, use a pipette to carefully resuspend the particles. Place the reagent container and mixing comb on the analyzer. Press the R. MIX button on the analyzer to start the motion of the mixing comb, if there is to be any delay in initiating processing.

2) Place the appropriate primary diluent line into the diluent container which is filled with PK CMV-PA SAMPLE DILUENT.

3) On the PK7200, remove the G stroke pins for the diluent lines only if a black rack with tubes of saline is not being processed at the beginning of the run.

4) Push the PREP button on the analyzer.

5) When the PREP cycle is complete, replace the G stroke pins, being sure to place the one marked "**G 0.25**" under the syringe that will be used to aspirate the PK CMV-PA SAMPLE DILUENT.

6) Press the DIAG button on the analyzer control panel to expel bubbles in the sample, reagent and diluted sample probes.

7) Proceed with sample analysis as described in the BECKMAN COULTER PK7200 Operator's Manual.

8) Please refer to Section XI., QUALITY CONTROL, for instructions about the use of control samples.

B. **PK7300 PREP Procedure for the BECKMAN COULTER PK CMV-PA SYSTEM :**

1) Place the reconstituted well mixed SENSITIZED PARTICLES into a PK7300 reagent vial. Place the reagent vial into the appropriate slot in the reagent tray. After the reagent tray is loaded into the PK7300, press the MIX button from the SYSTEM STATUS screen to mix the reagents

2) Place the appropriate diluent line into the diluent bottle which is filled with SAMPLE DILUENT.

3) Perform Diluent Priming by selecting the Preparation button on the SYSTEM STATUS screen.

4) Perform a Diluent and Reagent Check after selecting the Reagent/Diluent Status button from the SYSTEM STATUS screen.

5) Proceed with sample analysis as described in the BECKMAN COULTER PK7300 User's Guide.

6) Please refer to Section XI., QUALITY CONTROL, for instructions about the use of control samples.

XI. QUALITY CONTROL

The PK CMV-PA SYSTEM REACTIVE and NONREACTIVE CONTROLS should be tested at the beginning and end of each batch of samples assayed, after the addition of reagents and after interruption or delays in processing.

Refer to the PK CMV-PA SYSTEM CONTROLS package insert for complete details regarding this material. Additional quality control testing may be performed by the user including other well-characterized specimens or referenced sera.

Perform the test as described under Section X., DIRECTIONS FOR USE, using the reactive and nonreactive controls as specimens. The reactive control should produce a positive (+) reaction and the negative control should produce a negative (-) reaction with the test. If appropriate results are not obtained with the controls, all assay results within that batch are invalid and must be retested. Repeat testing making sure that the volume of the controls is sufficient for adequate instrument sampling (> 1.5 mL). When control material repeatedly fails to perform as expected, contact Beckman Coulter Immunohematology Technical Services at 800-447-5852.

XII. INTERPRETATION

The PK7200 and PK7300 will interpret the settling patterns of particles in each well based on the threshold setting chosen in the parameter file. See the PK7200 Standard Operating Procedure Manuals, or the PK7300 User's Guide, for complete details of the analyzer's interpretation of reactions.

Within 30 minutes of analyzer interpretation, on the PK7200, results should be verified by visual assessment of the reaction pattern against the photometric data. The PK7300 stores the reaction patterns on the hard drive and plate review may be performed either manually (within 30 minutes) or on-line (no time limit). Visually, a reactive test is a homogenous layer of particles. A nonreactive test would result in a compact, dense button surrounded by a clear zone. Plate review should include inspection of the reactions for abnormal settling patterns or for any sample for which visual and analyzer interpretations do not agree. Under certain circumstances, this homogenous layer of particles can be disrupted and fold over onto itself. An analogy would be the folding over of the sides of an omelet. This phenomenon can result from excessive vibration, protein buildup in the terraces of the microplate or physical damage to the microplate terraces, and is readily apparent during plate review. The recommended microplate maintenance procedures can be found in the Standard Operating Procedures (SOP) Manual for the PK

7200 or the PK7300 User's Guide.

A complete description of plate inspection and results review is contained in the Start-Up Procedures Section of the PK7200 SOP Manual and Section C of the PK7300 User's Guide. Additional testing must be performed on any samples for which visual and analyzer interpretations do not agree. Refer to the Analyzer Reaction Interpretation section of the PK7200 SOP Manual and Section C of the PK7300 User's Guide.

The presence or absence of antibody to cytomegalovirus is determined by the PK7200 and/or PK7300 using a CCD camera which analyzes the well image and can differentiate agglutinated and unagglutinated patterns. The PK7200 and/or PK7300 employs three assessment parameters for each microplate well containing PK CMV-PA SYSTEM reagent and test specimen:

- SPC Sharpness of the edge of the button
- LIA Quantity of particles in the center of the well
- P/C Ratio of the average light transmittance of the peripheral and central values

The parameters SPC, LIA and P/C are compared to programmable thresholds to obtain an interpretation (+,-,?) for each reaction.

The most important parameter resulting from the image analysis system is SPC. If the SPC is determined positive, then either a positive or indeterminate LIA or P/C value will result in an overall positive result interpretation for the reaction. A positive SPC value together with a negative value for either the LIA or P/C will cause the channel result to be indeterminate. If the SPC is determined negative, then either a negative or indeterminate LIA or P/C value will result in an overall negative result interpretation for the reaction. A negative SPC value together with a positive value for either the LIA or P/C will cause the channel result to be indeterminate.

Please refer to Table 1 for further clarification.

TABLE 1. DECISION LOGIC FOR PK7200 PHOTOMETRIC INTERPRETATION

Channel Result Interpretation	SPC	LIA	P/C
Positive	+	+ or ?	+ or ?
Negative	-	- or ?	- or ?
Indeterminate	+	-	-
	+	-	+
	+	+	-
	-	+	+
	-	+	-
	-	-	+
	?	+,-, or ?	+, -, or ?

XIII. INTERPRETATION OF RESULTS

A sample reported as nonreactive (-) on initial screening is considered negative for antibodies to CMV, indicating that the individual has not been infected with cytomegalovirus.

A sample reported as positive (+) on initial screening is considered reactive for antibodies to CMV by the criteria of the PK CMV-PA SYSTEM. The presence of antibodies indicates previous or current infection. Individuals with antibodies to CMV are potentially at risk of transmitting CMV infection, but are not necessarily contagious.

A sample reported as indeterminate (?) on initial screening may be considered reactive by the criteria of the PK CMV-PA SYSTEM, may be repeated in duplicate on the PK analyzer or tested by an alternative method.

If an initially indeterminate sample is repeated in duplicate using the PK CMV-PA SYSTEM, the duplicate tests must occur in the same run. If either duplicate is reactive or indeterminate, the specimen is to be interpreted as repeatedly reactive for antibodies to CMV by the criteria of the PK CMV-PA SYSTEM. If upon repeat testing both duplicate results are nonreactive, the sample should be considered negative for antibodies to CMV by the criteria of the PK CMV-PA SYSTEM.

Only those samples which test negative on initial screening or in both duplicate retests should be

considered negative for antibodies to CMV for purposes of transfusion.

XIV. LIMITATIONS OF THE PROCEDURE

The PK CMV-PA SYSTEM is used to detect circulating antibodies to cytomegalovirus. It has been shown to be safe and effective for the large scale screening of blood donors when used in accordance with instructions provided. Donors in the earliest stages of infection may not contain detectable levels of CMV antibody. The PK CMV-PA SYSTEM is not intended to distinguish between chronic and acute CMV infections.

This product is only for use in screening blood donors and has not been evaluated as a diagnostic test for CMV outside the blood bank setting.

XV. EXPECTED RESULTS

Several studies have shown the expected incidence of CMV antibodies in various populations. In a recent study of 250 random blood donors, 50% were positive for CMV antibodies.[3] This study supports earlier studies showing CMV antibody prevalence ranging from 20-82%.[2-4,18] Expected values may vary with age, sex and geographic location. The performance characteristics of the PK CMV-PA SYSTEM in blood donors were evaluated in two sites. Evaluation of 2020 blood donor samples demonstrate CMV seropositivity of 43.3%, 50.7%, for Sites 1 and 2, respectively.

XVI. SPECIFIC PERFORMANCE CHARACTERISTICS

PK7200

The performance of the PK CMV-PA SYSTEM(PK CMV-PA) was compared to other serologic assays for CMV antibody activity in two phases. In phase 1 (Tables 2-3), a total of 2020 random donor samples were evaluated in two geographically distinct blood centers. The particle agglutination assay, PK CMV-PA, was compared to the PK CMV hemagglutination assay(PK CMV-HA) at both sites. Assays were performed using the PK7200 Automated Microplate System. In addition, each site also compared the PK CMV-PA SYSTEM to either a passive latex agglutination (LA) assay or an enzyme immunoassay (EIA) for total CMV antibody. Samples with indeterminate results were repeated in duplicate with the corresponding assay. Samples that were discordant among the three assays being performed at each site were also repeated in duplicate with each assay. Samples that continued to be discordant were further evaluated by additional supplemental testing, microparticle enzyme immunoassay (MEIA) and EIA or LA, depending on the matrix of the original site comparison. Results were considered concordant if three of the five assay results agreed.

In phase 2 (Table 4), one hundred (100) samples characterized for CMV serostatus, were evaluated by each of the two sites. This cohort included sixty (60) CMV positive samples ranging from low to high reactivity and forty (40) CMV nonreactive samples.

Reproducibility

The reproducibility of the PK CMV-PA SYSTEM was evaluated at the two sites detailed above. Using the PK7200 analyzers, a cohort of fifty (50) samples characterized for CMV serostatus, was tested on three (3) days against three (3) reagent lots of PK CMV-PA. The sample cohort included nonreactives and reactives with a broad range of antibody activity. Results were in agreement for 1778 of 1800 (98.7%) possible data points (900 data points were from testing on two PK7100's). No CMV reactive samples gave negative results. All twenty-two (22) data points that were discordant occurred when CMV nonreactive samples tested positive or indeterminate. 15 (68.1%) of the 22 discordant results occurred with one CMV nonreactive sample, pointing to suspect sample condition.

Clinical Specificity

The PK CMV-PA SYSTEM was tested with a cohort of 41 CMV negative samples from individuals demonstrating reactivity for rheumatoid factor, infectious mononucleosis, rubella, herpes simplex virus, Epstein-Barr virus, antinuclear antibodies, mycoplasma pneumonia, and hepatitis B surface antigen. No evidence of interference or crossreactivity with PK CMV-PA was observed with the exception of one of six positive or indeterminate (?) samples. Thirty-five

(35) samples were negative with the PK CMV-PA assay and were not further tested by Latex or EIA. Two (2) Rheumatoid Factor samples and one (1) ANA sample produced positive (+) results with the PK CMV-PA assay, with positive (+) agreement by CMV Total EIA and PK CMV Hemagglutination. Therefore, it is assumed that the original assay was incorrect. One RF sample, which was reactive with the PK CMV-PA and EIA assays but nonreactive (-) with the PK CMV assay and the original CMV assay, is considered discrepant and unresolved. One (1) HAV and (1) rheumatoid factor sample produced an indeterminate (?) on the PK7200 using both the PK CMV-PA and the PK CMV hemagglutination assay, but negative with EIA and positive with latex. Therefore, the PK7200 indeterminate results for these two samples may be considered discrepant and unresolved. Nine (9) mononucleosis samples demonstrated nonreactivity with the PK CMV-PA but tested reactive with the Latex test. In eight (8 of 9) of these cases the EIA agreed with the PK CMV-PA nonreactive results but was positive with one (1 of 9) sample. Based on these results, the PK CMV-PA test demonstrated no true reactivity with samples containing potentially crossreactive disease states when compared to the PK CMV Hemagglutination assay and a commercially marketed Total CMV EIA test. However, it must be considered that certain RF or HSV patient samples may produce false positive or indeterminate results on the PK7200 with agglutination assays.

PK7300

The performance of the PK CMV-PA SYSTEM was evaluated on the PK7300 by comparing to the PK7200 reference results. Testing was performed at two geographically distinct blood centers and also at Olympus America, Inc. A total of 2983 serum samples and 7394 plasma (EDTA) samples were tested. Results are summarizes in Table 5.

Reproducibillity

The reproducibillity of the PK CMV-PA SYSTEM on the PK7300 was evaluated by testing a subset, 1154 plasma samples, of the total samples tested in the study. Included in the subset were 27 known reactive and 4 known non-reactive samples. All samples were tested on days 1-3, 4 and 6. Results are summarized in Table 6 and 7.

Clinical Specificity

The PK CMV-PA SYSTEM was tested with a cohort of 7 CMV negative samples on the PK7300 from individuals demonstrating reactivity for antinuclear antibodies, rheumatoid factor and Lyme's disease. All samples tested negative as expected with no evidence of interference or crossreactivity.

Sensitivity and Specificity

Sensivity and Specificity for the PK CMV-PA SYSTEM when tested on the PK7300 was determined by comparing the 2983 serum samples and the 7394 plasma samples from field trial testing to the "true" result obtained on the PK7200. Discordant samples were tested by two other methods (EIA and Capture-R). Best two out of three results was considered the "true" result after additional testing. Results are summarized in Tables 8 and 9.

TABLE 2. SITE 1: COMPARISON OF PK CMV-PA TO EIA USING THE PK7200 WITH RANDOM DONORS

PK CMV-PA	TOTAL CMV EIA	Initial	Repeat*	After resolution of discordants**
N	N	569	572	572
R	R	432	433	433
R	N	4	4	4
N	R	1	1	1
I	N	3		
I	R	1		
Total		1010	1010	1010

% CONCORDANCE 1005/1010 = 99.5%

R = reactive N = nonreactive I = indeterminate

* Repeat testing of initial indeterminates only

** Discordants resolved by agreement of 3 of 5 assays (PK CMV-PA, PK CMV, EIA, LA and MEIA)

TABLE 3. SITE 2: COMPARISON OF PK CMV-PA TO LATEX AGGLUTINATION USING THE PK7200 WITH RANDOM DONORS

PK CMV-PA	LATEX AGGL	Initial	Repeat*	After resolution of discordants**
N	N	489	493	493
R	R	496	498	498
R	N	14	14	14
N	R	5	5	5
I	R	1		
R	I	1		
I	N	2		
N	I	2		
Total		1010	1010	1010

% CONCORDANCE 991/1010 = 98.1%

R = reactive N = nonreactive I = indeterminate

* Repeat testing of initial indeterminates only

** Discordants resolved by agreement of 3 of 5 assays (PK CMV-PA, PK CMV, EIA, LA and MEIA)

TABLE 4. PK CMV-PA RESULTS FOR A COHORT OF CHARACTERIZED SAMPLES

	CMV CHARACTERIZED			
	Site 1(PK7200)		Site 2(PK7200)	
PK CMV-PA	R	N	R	N
R	60	1*	60	0
N	0	39	0	40
Totals	60	40	60	40
% Agreement	99/100 = 99%		100/100 = 100%	
Relative Sensitivity	60/60 = 100%		60/60 = 100%	
Relative Specificity	39/40 = 97.5%		40/40 = 100%	

* same sample

TABLE 5. INITIAL AND REPEAT CMV RESULTS WITH PLASMA AND SERUM

PK-CMV-PA SYSTEM		PLASMA		SERUM	
PK7200	PK7300	Initial	Repeat	Initial	Repeat
Neg	Neg	3340	3365	1455	1462
Neg	Pos	19	2	1	0
Pos	Neg	15	1	11	2
Pos	Pos	4020	4026	1516	1519
Totals		7394	7394	2983	2983
% CONCORDANCE		99.50%	99.9%	99.6%	99.9%

TABLE 6. RATE OF AGREEMENT FOR SAMPLE AGE SUBSET ON THE PK7300

Test Day	Number In Agreement	Rate of Agreement(%)	Lower 95% confidence bound
Initial	1143/1154	99.05%	98.43%
Day 4	1144/1154	99.13%	98.53%
Day 6	1146/1154	99.31%	98.75%

TABLE 7. REPRODUCIBILITY OF THE PK CMV-PA SYSTEM ON THE PK7300

	N =	DAY 1-3	DAY 4	DAY 6
Known Reactive	81	81	76	81
Known Non-Reactive	12	11**	11**	11**

* 5 reactive and 1 non-reactive samples were omitted from testing in error.
** 1 sample tested faise positive on day 1-3 and 6.

TABLE 8. SENSITIVITY FOR CMV REAGENT

Sample Type	Correct Result Positive	Incorrect Negative Result	Sensitivity	Sensitivity % (95% lower Conf. Bound)
Serum	1517	4	1517/1521 99.74%	99.40%
EDTA Plasma	4026	0	4026/4026 100.0%	99.93%

TABLE 9. SPECIFICITY FOR CMV REAGENT

Sample Type	Correct Result Negative	Incorrect Positive Result	Specificity	Specificity % (95% lower Conf. Bound)
Serum	1462	0	1462/1462 100.0%	99.80%
EDTA Plasma	3355	13	3355/3368 99.61%	99.39%

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