AccuDiag™ Cytomegalovirus IgG (CMV IgG) ELISA Kit

REF 1201-P1

IVD See External Label 2-8°C 96 Tests

CMV IgG ELISA	
Principle	Indirect ELISA
Detection	Qualitative and Semiquantitative
Sample	10 µL serum/plasma
Incubation Time	75 minutes
Sensitivity	99.2%
Specificity	94.1%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. AccuDiag™ Cytomegalovirus (CMV) IgG Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection and semiquantitative determination of IgG antibody to cytomegalovirus in human sera to indicate previous infection with cytomegalovirus. Paired sera, acute and convalescent, may be used to demonstrate seroconversion or a significant rise in antibody level, as an aid in the diagnosis of a recent or current infection, or reactivation. This product is not FDA cleared (approved) for use in testing, ie., screening, blood or plasma donors. **For in vitro diagnostic Use. High complexity test.**

SIGNIFICANCE AND SUMMARY

Throughout most of the world, cytomegalovirus (CMV) infection is asymptomatically acquired during childhood. However, in affluent communities, primary infection may be delayed resulting in:

- infection during pregnancy which may lead to overt or delayed onset of congenital abnormalities in the newborn,
- infection following blood transfusions which may lead to CMV induced mononucleosis,
- infection following immunosuppression for organ transplantation which may lead to complications during recovery and/or loss of the organ.

Infection by CMV cannot be clinically diagnosed without confirmation by laboratory testing such as isolation of the virus or the demonstration of a significant rise in specific antibody level.

In 1904 Rippert described the large inclusion containing cells which are CMV's primary anatomic pathologic effect.[1,2] This herpesvirus was first isolated fifty years later by Smith but the descriptive term cytomegalovirus was coined by Weller.[3] CMV has the capacity to persist in its human host indefinitely as a latent infection in several glands and the kidneys. Unlike the other herpesviruses, CMV is slow growing, producing a delayed cytopathic effect in cell culture. Cytomegaly is characteristic of a CMV infection resulting in swollen cells containing large paranuclear inclusions.

Prevalence studies based on the frequency of seropositive individuals in the general population (40-100%), shows inverse correlation between the acquisition of CMV infection and the socioeconomic condition of the population. Age-related incidence studies suggest increased risk of infection during both the perinatal and reproductive periods of the human lifecycle.[4] Perinatal infection can be acquired through cervical secretions and breast milk while the sudden increase in seroconversion at sexual maturity is suggestive of a possible venereal transmission.

Though less frequent, prenatal CMV infection may result from transplacental transmission from mother to fetus and is the major infectious cause of mental retardation and other congenital defects in the newborn. Only 1 in 2,000 infants are born expressing the severe cytomegalic inclusion disease (CID), while ten times that many acquire an asymptomatic infection in utero. Medically, the asymptomatic or "silent" congenital disease, is important because of possible longterm developmental effects and the lack of overt clinical signs to guide the physician's diagnosis.[5]

Additionally, two types of iatrogenic infection can occur. First, a recurrent or reactivated infection frequently follows the immunosuppressive therapy which typically accompanies organ transplantation [6] or cancer treatment.[7] Second, recipients of multiple transfusions usually acquire either a primary or reactivated infection.[8] These opportunistic infections are frequently subclinical but the severity of the disease state depends on the dose received and the immune status and competence of the individual's immune system.

Since the presence of circulating IgG antibody to CMV is indicative of previous infection, screening pregnant women, organ transplant recipients and other immunosuppressed patients for seropositivity is a valuable tool for determining whether or not they have been previously infected.

As first described by Engvall and Perlman [9,10,11] and Van Weeman, [12] Enzyme Immunoassays are both specific and sensitive for the detection and measurement of serum proteins. The sensitivity, specificity, and reproducibility of enzyme-linked immunoassays is comparable to other serological tests for antibody, such as immunofluorescence (IFA),

complement fixation, hemagglutination and radioimmunoassay.[13,14,15,16] Recently enzyme immunoassays have been developed for the detection of antibody to cytomegalovirus and shown to be useful.[15]

The Diagnostic Automation Inc. Cytomegalovirus IgG ELISA kit provides all the necessary reagents for the rapid semi-quantitative determination of IgG antibody to cytomegalovirus in human sera.

ASSAY PRINCIPLE

Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO_4, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.[9,10,11,12]

SPECIMEN COLLECTION & PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the Diagnostic Automation Inc. ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, nonlipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.[23] Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2° and 8°C if testing will take place within two days. If specimens are to be kept for longer periods, store at -20°C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. If paired sera are to be collected, acute samples should be collected as soon as possible after the onset of symptoms. The second sample should be collected 14 to 21 days after the acute specimen was collected. Both samples must be run in duplicate on the same plate to test for a significant rise. If the first specimen is obtained late during the course of the infection, a significant rise may not be detectable.
5. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).[23]

REAGENTS

Materials provided with the kit

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. Cytomegalovirus antigen (inactivated) coated micro assay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)
2. Serum Diluent Type I: Ready for use. Contains ProClin® (0.1%) as a preservative. (96T: one bottle, 30 mL)
3. Calibrator: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T: one vial, 0.4 mL) *
4. Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Positive Control is utilized to control the positive range of the assay. (96T: one vial, 0.4 mL) *
5. Negative Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label.The Negative Control is utilized to control the negative range of the assay. (96T: one vial) *
6. Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgG, containing ProClin® (0.1%) and gentamicin as preservatives. (96T: one bottle, 16 mL)
7. Chromogen/Substrate Solution Type I: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 mL)
8. Wash Buffer Type I (20X concentrate): Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and ProClin® (0.1%) as a preservative. (96T: one bottle, 50 mL)
9. Stop Solution: Ready to use, contains a 1N H2SO4 solution. (96T: one bottle, 15 mL)

***Note: serum vials may contain excess volume.**

Materials required but not provided

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
3. One-liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water (dH20), CAP (College of American Pathology) Type 1 or equivalent.[25, 26]
9. Timer capable of measuring to an accuracy of +/- 1 second (0 - 60 minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH_2O).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware.

REAGENT PREPARATION

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21° to 25°C). Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H20. Mix well.

ASSAY PROCEDURE

Note: To evaluate paired sera, both serum samples must be tested in duplicate and run in the same plate. It is recommended that the serum pairs be run in adjacent wells.

1. Place the desired number of strips into a microwell frame. Allow four (4) Control/Calibrator determinations (one Negative Control, two Calibrators and one Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient #4
1B	NC	2B	Patient #5
1C	Cal	2C	Patient #6
1D	Cal	2D	Patient #7
1E	PC	2E	Patient #8 (Acute 1)
1F	Patient #1	2F	Patient #8 (Acute 2)
1G	Patient #2	2G	Patient #8 (Convalescent 1)
1H	Patient #3	2H	Patient #8 (Convalescent 2)

RB = Reagent Blank – Well without serum addition run with all reagents. Utilized to blank reader.
NC = Negative Control
Cal = Calibrator
PC = Positive Control

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µL + 200 µL) in Serum Diluent. Mix well. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum.)
3. To individual wells, add 100 µL of the appropriate diluted Calibrator, Controls and patient sera. Add 100 µL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21° to 25°C) for **25 minutes +/- 5 minutes.**
5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.
   ****IMPORTANT NOTE:** Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semiautomated or automated equipment set to deliver a volume to completely fill each well (250-300µL) is recommended. A total of up to five (5) washes may be necessary with automated equipment. **Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.**
6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21° to 25°C) for **25 minutes +/- 5 minutes.**
8. Repeat wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21° to 25°C) for **10-15 minutes.**
11. Stop reaction by addition of 100 µL of Stop Solution (1N H_2SO_4) following the same order of Chromogen/Substrate addition, including the reagent blank well.Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is ≥ 0.150 the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

RESULTS

CALCULATIONS

1. Mean Calibrator O.D. (Optical Density) - Calculate the mean O.D. value from the two Calibrator determinations.
2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by Diagnostic Automation Inc. for each lot of kits. The Correction Factor is printed on the Calibrator vial.
3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.
4. ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cutoff Calibrator Value determined in Step 3.

Example:

O.D's obtained for Calibrator	= 0.38, 0.42
Mean O.D for Calibrator	= 0.40
Correction Factor	= 0.50
Cutoff Calibrator Value	= 0.50 x 0.40 = 0.20
O.D. obtained for patient sera	= 0.60
ISR Value	= 0.60/0.20 = 3.00

INTERPRETATION

ANALYSIS

1. The patients' ISR (Immune Status Ratio) values are interpreted as follows:

ISR	Results	Interpretation
≤ 0.90	**Negative**	**NO detectable antibody to Cytomegalovirus by the ELISA test. Such individuals are presumed to be uninfected with CMV and to be susceptible to primary infection.**
0.91 – 1.09	**Equivocal**	**Samples should be retested. See Number (2) below.**
≥ 1.10	**Positive**	**Indicates presence of detectable antibody to Cytomegalovirus by the ELISA test. Indicative of current or previuos infection. The**

ISR	Results	Interpretation
		individual may be at risk of transmitting CMV infection, but is not necessarily currently contagious.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.
3. In the evaluation of paired sera, if the acute specimen is negative and the convalescent specimen is positive, a seroconversion has taken place. This indicates a significant change in antibody level and the patient is undergoing a primary infection.
4. To evaluate paired sera for a significant change in antibody level or seroconversion, both samples must be tested in duplicate in the same assay. The mean ISR of both samples (acute and convalescent) must be greater than 1.00 to evaluate the paired sera for significant rise in antibody level.
5. Additional Quality Control for Paired Sera: (See NOTE under Assay Procedure). As a check for acceptable reproducibility of both the acute sera (tested in duplicate) and the convalescent sera (tested in duplicate), the following criteria must be met for valid results:

Acute 1 ISR = 0.8 to 1.2 **Convalescent 1 ISR = 0.8 to 1.2**
Acute 2 ISR **Convalescent 2 ISR**

6. Compare the ISR of the pairs by calculating as follows:

$$\frac{\text{Mean ISR (second sample)} - \text{Mean ISR (first sample)}}{\text{Mean ISR (first sample)}} \times 100 = \%\ \text{RISE IN ISR LEVEL}$$

% Rise In ISR	Interpretation
< 30.0%	No significant change in antibody level. No evidence of recent infection. If active disease is still suspected, a third sample should be collected and tested in the same assay as the first sample to look for a significant rise in antibody level.
≥ 30.0%	Statistically significant change in antibody level detected. This identifies those persons who are presumed to be experiencing recent or current episodes of CMV infection (reactivation, reinfection or a primary infection where the acute specimen was obtained too late to demonstrate seroconversion.

Note: *When evaluating paired sera, it should be determined if samples with high absorbance values are within linearity specifications of the spectrophotometer. Read the Operator's Manual or contact the instrument's manufacturer to obtain the established linearity specifications of your spectrophotometer.*

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:
1. Calibrator and Controls must be run with each test run.
2. Reagent blank (when read against air blank) must be < 0.150 Absorbance (A) at 450 nm.
3. Negative Control must be ≤ 0.250 A at 450 nm (when read against reagent blank).
4. Each Calibrator must be ≥ 0.250 A at 450 nm (when read against reagent blank).
5. Positive Control must be ≥ 0.500 A at 450 nm (when read against reagent blank).
6. The ISR (Immune Status Ratio) Values for the Positive and Negative Control should be in their respective ranges printed on the vials. If the Control values are not within their respective ranges, the test should be considered invalid and should be repeated.
7. Additional Controls may be tested according to guidelines, or requirements of local, state, and/or federal regulations or accrediting organizations.
8. Refer to NCCLS C24-A for guidance on appropriate QC practices.[24]
9. If above criteria are not met upon repeat testing, contact Diagnostic Automation Inc. Technical Services.

EXPECTED RANGES OF VALUES

Two hundred random samples collected from a large Washington, D.C. metropolitan hospital were tested for CMV IgG antibodies using the Diagnostic Automation Inc. CMV IgG ELISA product. Serologic status analysis from this study indicates that a large percentage of these individuals have had previous infection with CMV. Results of this analysis are in the following table:

Serologic Status Analysis

% of Total	Negative	Equivocal	Positive
(200 samples)	25%	9%	66%

Several studies suggest a relationship between CMV IgG antibody incidence and age, [8,20] socioeconomic status, [21] and geographic location of the population tested.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

A total of 200 random samples from a large Washington, D. C. hospital population were assayed with the Diagnostic Automation Inc. CMV IgG ELISA and with a second commercially available ELISA Test Kit. The study population was composed of individuals from a transplantation serology clinic and randomly collected sera from normal donors. A commercially available IFA antibody test for CMV IgG was used in all cases of discordant positive or negative results. The results of this study were as follows:

	Diagnostic Automation Inc. ELISA				
IFA and ELISA		+	–	Relative Sensitivity	Relative Specificity
	+	130	1	99.2%	94.1%
	–	3	48	(130/131)	(48/51)

Equivocal by both ELISA methods were considered indeterminate and these results (18 total) were omitted from the calculations for relative sensitivity and specificity.

REPRODUCIBILITY

Three studies were performed to assess the precision on the TMB test results. Five sera were used in 20 wells each for the intra-run assay. Five sera were used for 5 days in 5 wells each for the inter-day assay results. Five sera were used in 3 wells each ranging from negative to high positive with 3 different lots of TMB substrate. The results are as follows:

Table I
INTRA-RUN ASSAY

		Serum 1	Serum 2	Serum 3	Serum 4	Serum 5
Mean	=	2.91	1.80	1.00	0.781	0.239
S.D.	=	0.21	0.097	0.061	0.043	0.012
C.V.	=	7.4%	5.4%	6.1%	5.5%	5.1%

Table II
INTER-DAY

		Serum 1	Serum 2	Serum 3	Serum 4	Serum 5
Mean	=	3.05	1.71	0.99	0.21	0.78
S.D.	=	0.196	0.094	0.045	0.016	0.02
C.V.	=	6.4%	5.5%	4.6%	7.7%	3.0%

Table III
INTER-LOT ASSAY

		Serum 1	Serum 2	Serum 3	Serum 4	Serum 5
Mean	=	3.84	2.77	1.58	0.43	1.73
S.D.	=	0.414	0.365	0.265	0.073	0.337
C.V.	=	10.8%	13.2%	16.8%	16.9%	19.5%

Intra-Assay Precision
Table II presents the results of four (4) samples individually pipetted in groups of twenty (20) in a single assay.

Table II
Intra-Assay Precision for Varicella-zoster IgG

	N	Mean ISR	Std Dev	%CV
Serum 1	20	2.91	0.202	7.0%
Serum 2	20	4.99	0.184	3.7%
Serum 3	20	0.84	0.154	18.3%
Serum 4	20	0.53	0.067	12.8%

Inter-Assay Precision
Table III presents the summary of the Inter-Assay precision data determined by replicate testing of four (4) samples individually pipetted in groups of five (5) on three (3) consecutive days.

Table III
Inter-Assay Precision for Varicella-zoster IgG

	Day 1	Day 2	Day 3	n	Mean ISR	Std Dev	%CV
Serum 1	2.57	2.41	2.89	3	2.62	0.220	8.4%
Serum 2	4.24	4.01	4.61	3	4.29	0.267	6.3%
Serum 3	0.77	0.84	0.97	3	0.86	0.096	11.2%
Serum 4	0.38	0.52	0.40	3	0.43	0.070	16.3%

PERCENT RISE IN ISR
A study was conducted to evaluate the percent rise in ISR between simulated paired sera (acute and convalescent) where a significant rise in antibody level would not be expected. Ten sera were diluted for assay twice and then evaluated as paired sera. The results of the study show the calculated percent rise in ISR between the simulated paired sera to be 7.2% or less.

A second study was conducted using actual documented clinical acute and convalescent sera. All specimens were tested by the Diagnostic Automation Inc. CMV IgG ELISA and an IFA test. Four of the paired sera were also test by a latex agglutination test. The results of this study are presented in Table IV:

Table IV

Sample	ISR	% Rise in ISR	IFA Titer	Rise in Titer	Latex Aggl Titer	Rise in Titer
1A	2.1		16		--	
1C	2.5	22	32	2 Fold		
2A	1.1		16		--	
2C	4.5	313	256	16 Fold	--	
3A	1.5		16		--	
3C	3.9	166	4096	250 Fold	--	
4A	2.3		256		8	
4C	4.3	93	512	2 Fold	64	8 Fold
5A	2.9		512		16	
5C	6.3	121	16384	32 Fold	256	16 Fold
6A	3.8		256		16	
6C	5.4	44	512	2 Fold	64	4 Fold
7A	3.9		128		16	
7C	5.1	30	256	2 Fold	64	4 Fold

Note: A = Acute, C = Convalescent
Sample 1A was a seroconversion by indirect hemagglutination (IHA)

CROSS-REACTIVITY STUDY
Method
A study was performed to determine the cross-reactivity of the Diagnostic Automation Inc. CMV IgG ELISA with samples which tested negative by IFA for CMV IgG, but positive by IFA for HSV-1 IgG (3), HSV-2 IgG (3), VZV IgG (3) and ANA (4).

Results
Negative Diagnostic Automation Inc. CMV IgG ELISA test results in all five samples indicate an absence of cross-reactivity of the Diagnostic Automation Inc. CMV IgG ELISA with ANA and the other members of the Herpes virus family.

LIMITATIONS OF THE ASSAY

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps and control of incubation temperature are essential for accurate results.
2. This kit is designed to measure IgG antibody in patient samples. Positive results in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the fetus before birth. IgM assays are generally more useful indicators of infection in children below the age of six months.
3. A significant rise in antibody level indicates recent antigenic stimulation but does not necessarily indicate active viral excretion. Since fourfold fluctuations in CMV IgG antibody levels have been noted in some apparently healthy seropositive individuals, the most definitive means of diagnosing active CMV infection requires viral culture. However, asymptomatic viremia has also been described.[18,19,22]
4. Lack of a significant rise in antibody does not exclude the possibility of cytomegalovirus infection.
5. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended that an IgM assay be performed, or a second serum sample be obtained at 10 - 21 days later to be tested in parallel with the original sample to determine seroconversion, which is indicative of a primary infection.
6. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.
7. The presence of CMV IgG antibody may not assure protection from disease.
8. The results of a single specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.

9. The values obtained from this assay are intended to be an aid to diagnosis only. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures.

STORAGE CONDITIONS

1. Store unopened kit between 2° and 8°C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2° and 8°C. Unused strips must be immediately resealed in a sealable bag with desiccant and returned to storage between 2° and 8°C.
3. Store HRP Conjugate between 2° and 8°C.
4. Store the Calibrator, Positive Control, and Negative Control between 2° and 8°C.
5. Store Serum Diluent Type I and 20X Wash Buffer Type I between 2° and 8°C.
6. Store the Chromogen/Substrate Solution Type I between 2° and 8° C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (diluted) Wash Buffer Type I at room temperature (21° to 25°C) for up to 5 days, or up to one week between 2° and 8°C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination.

PRECAUTIONS

1. **For in vitro diagnostic use.**
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2. 17
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type I, and Serum Diluent Type I. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21° to 25°C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may occur**.
7. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. **Cross-contamination of reagents and/or samples could cause false results**.
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. **Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents**.
13. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing ProClin®, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.
15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with water.
17. **Caution**: Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. The concentrations of anti-Cytomegalovirus in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

The safety data sheet is available upon request.

WARNING

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300R, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P302 + **P352**: IF ON SKIN: Wash with plenty of soap and water.
P333 + **P313**: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Serum Diluent and Controls contain < 0.1% sodium azide.
H302: Harmful if swallowed
P264: Wash thoroughly with plenty of soap and water after handling
P270: Do not eat, drink or smoke when using this product
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
P330: If swallowed, rinse mouth
P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

REFERENCES

1. Rippert, H. 1904.Verber protozoenartige Zellen in der Niere eines syphilitischen Neugeborenen und in der Parotis von Kindern. Centralb. g Allg. Pathol. 15: 945-948.
2. Smith, M. G. 1954. Propagation of Salivary gland Virus of the Mouse in Tissue Cultures. Proc. Soc. Exp. Biol. Med. 86: 435-440.
3. Weller, T. H., J. B. Hanshaw and D. E. Scott. 1960. Serologic Differentiation of Viruses Responsible for Cytomegalomic Inclusion Disease. Virology. 12: 130-132.
4. Carlstrom, G. and B. Jalling. 1970. Cytomegalovirus Infections in Different Groups of Paediatric Patients. Acta Paediatr. Scand. 59: 303-309.
5. Melish, M. E. and J. B. Hanshaw. 1973. Congenital Cytomegalovirus Infection: Developmental Progress of Infants Detected by Routine Screening. Am. J. Dis. Child. 126: 190-194.
6. Ho, M. 1982. Cytomegalovirus. In: Biology and Infection. NY. Plenum Medical Book Co.
7. Bodey, G. P., P. T. Wertlake, G. Douglas, et al. 1965. Cytomegalic Inclusion Disease in Patients with Acute Leukemia. Ann. Intern. Med. 62: 899-906.
8. Prince, A. M., W. Szmuness, S. J. Millian, et. al. 1971. A Serologic Study of Cytomegalovirus Infections Associated with Blood Transfusions. N. Engl. J. Med. 284: 1125-1131.
9. Engvall, E., and P. Perlman. 1971. Enzyme-Linked Immunosorbent Assay, (ELISA) Quantitative Assay of Immunoglobulin G. Immunochemistry. 8: 871-874.
10. Engvall, E., and P. Perlman. 1971. Enzyme-Linked Immunosorbent Assay, ELISA. Peeters. H., ed. In: Protides of the Biological Fluids. Proceedings of the Nineteenth Colloquium, Brugge, Oxford. Pergamon Press. pp 553-556.
11. Engvall, E., K. Jonsson, and P. Perlman. 1971. Enzyme Linked Immunosorbent Assay. II. Quantitative Assay of Protein Antigen, Immunoglobulin-G, By Means of Enzyme- Labelled Antigen and Antibody-Coated tubes. Biochem. Biophys. Acta. 251: 427-434.
12. Van Weeman, B. K. and A. H. W. M. Schuurs. 1971. Immunoassay Using AntigenEnzyme Conjugates. FEBS Letter. 15: 232-235.
13. Bakerman, S. 1980. Enzyme Immunoassays. Lab. Mgmt. August: 21-29.
14. Booth, J. C., G. Hannington, T. A. G. Ariz and H. Stern. 1979. Comparison of enzymelinked immunosorbent assay (ELISA) Technique and complement-fixation test for estimation of cytomegalovirus IgG antibody. J. Clin. Pathol. 32: 122-127.
15. Cappel, R., F. De Cuyper and J. De Braekeleer. 1978. Rapid detection of IgG and IgM antibodies for cytomegalovirus by the enzyme-linked immunosorbent assay (ELISA). Arch. Virol. 58: 253-258.
16. Engvall, E. and P. Perlman. 1972. Enzyme-Linked Immunoasorbent Assay ELISA. III. *Quantitation of Specific Antibodies by Enzyme-Labelled Anti-Immunoglobulins in Antigen-Coated Tubes. J. Immunol. 109: 129-135.
17. CDC-NIH Manual. 1993. In: Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition. U. S. Dept. of Health and Human Services, Public Health Service. pp 9-12.
18. Starr, S. E. and H. M. Friedman. 1985. Human Cytomegalovirus. In: Manual of Clinical Microbiology, Fourth edition. E. H. Lennette, A. Balows, William J. Hausler, Jr. and M. J. Shadomy, eds. American Society for Microbiology 65: 711-719.
19. Warner, J. L., T. H. Weller, and S. V. Kevy, 1973. Patterns of Cytomegaloviral Complement-Fixing Antibody Activity: A Longitudinal Study of Blood Donor. J. Infect. Dis. 127: 538-543.
20. Wentworth, B., and E. R. Alexander. 1971. Seroepidemiology of Infections Due to Members of the Herpes Virus Group. Am . J. Epidemiol. 94: 496-507.
21. Stagno. S., R. F. Pass, M. E. Dworskey, R. E. Henderson, E. G. Moore, P. D. Walton, and C. A. Alford. 1982. Congenital Cytomegalovirus Infection: The Relative Importance of Primary and Recurrent Maternal Infection. N. Engl. J. Med. 306: 945-949.
22. Cox. F. and W.T. Hughes. 1975. J. Pediatrics. 87: 190-194.
23. National Committee for Clinical Laboratory Standards. 1990. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture Approved Standard. NCCLS Publication H18-A.
24. NCCLS. 1991. National Committee for Clinical Laboratory Standard. Internal Quality Control Testing: Principles & Definition. NCCLS Publication C24- A.
25. http://www.cap.org/html/ftpdirectory/checklistftp.html. 1998. Laboratory General - CAP (College of American Pathology) Checklist (April 1998). pp 28-32.
26. NCCLS. 1997. National Committee for Clinical Laboratory Standard. Preparation and Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3-A3.

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

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Diagnostic Automation/Cortez Diagnostics, Inc.
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Woodland Hills, California 91367 USA

Date Adopted	**2023-09**
Brand Name	**AccuDiag™**
REF 1201-P1	**AccuDiag™ - Cytomegalovirus IgG (CMV IgG) ELISA**
Revision Date: 2019-10-31	