

Preliminary Evaluation of the Abbott ARCHITECT® Anti-Cytomegalovirus IgG*, IgM* and IgG Avidity* Assays Linked by an Automated Reflex Algorithm

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American Association for Clinical Chemistry Annual Meeting • Chicago, Illinois • July 23 – 27, 2006

Abstract (Revised)

Objectives

Development and preliminary evaluation of a completely automated panel of Cytomegalovirus (CMV) IgG, IgM and IgG avidity assays that are linked by a user-editable automated reflex algorithm.

Methods

The three CMV assays for the ARCHITECT instrument are two-step immunoassays utilizing CMV virus lysate-coated paramagnetic microparticles for the capture of human anti-CMV antibodies. The CMV IgM assay contains both viral lysate and the recombinant protein CKS-pp150, pp52 (UL32, UL44) coated onto paramagnetic particles. An acridinium-labeled monoclonal antibody directed against human IgG or IgM was utilized for human anti-CMV antibody detection. The CMV IgG avidity test requires dilution of the patient sample based upon the CMV IgG antibody titer. A user-editable automated reflex algorithm has been developed that orders the CMV-IgM assay and the CMV IgG avidity assay when the sample fulfills predefined criteria. In the first part of the study the performance of the ARCHITECT CMV IgG and IgM assays was compared to the Abbott AxSYM CMV assays by testing patient samples from pregnant women, blood donors, transplant recipients and seroconversion panels. Samples with discrepant results were tested on two additional CMV IgG or CMV IgM assays and resolved with the consensus. The performance of the ARCHITECT CMV IgG avidity assay was evaluated by comparison to the Radim CMV IgG Avidity assay and clinical information. In the second part of the study, patient samples from blood donors, hospitalized patients and pregnant women were tested with the panel of ARCHITECT CMV IgG, IgM and IgG avidity assays using an automated reflex algorithm, whereby the instrument automatically orders the CMV IgM test if the CMV IgG test result is positive and the CMV IgG avidity test if both the CMV IgG and IgM test results are positive.

Results

In the first part of the study, the ARCHITECT CMV IgG assay had a resolved relative sensitivity of 100% and a resolved relative specificity of 99.5% when compared to AxSYM CMV IgG. The resolved specificity of the CMV IgM assay was 99.0%. The ARCHITECT CMV IgG avidity assay using "AVIcomp" technology displayed 99.6% clinical specificity on CMV immune blood donors and pregnant women. The clinical sensitivity of the avidity assay was 97% on seroconversion panels. The clinical sensitivity and specificity of the ARCHITECT CMV IgG avidity were superior to the reference avidity assay. In the second part of the study, 1,249 samples from blood donors, pregnant women and hospitalized patients were tested by the CMV IgG assay. Of these, 583 samples (46.7%) were CMV IgG positive and were reflex tested by the CMV IgM assay. Of these, 37 were both CMV-IgG and CMV-IgM positive and were reflex tested by the CMV IgG avidity assay. Three of these samples contained low avidity IgG.

Conclusion

The performance of the ARCHITECT CMV IgG avidity assay was superior to the Radim CMV IgG assay. The ARCHITECT instrument offers complete automation of the CMV panel including avidity with the reflex test option available on the *i2000_{SR}* and *i1000** instruments. Three automated CMV testing algorithms have been developed for the CMV Panel on the ARCHITECT instrument that can be edited by the user, thus allowing adaptation of the retest rules to specific requirements of the laboratory.

Introduction

Human Cytomegalovirus (CMV) is a herpesvirus that commonly infects the human population. CMV is the most common congenital infection, occurring in approximately 1% of all live births. Intrauterine transmission of primary CMV infection, especially during the first trimester, is the second leading cause of mental retardation after Down's syndrome. Since CMV IgM antibodies can be produced during primary and non-primary CMV infections, CMV IgG avidity tests have been found useful at the

diagnostic level to discriminate between primary and non-primary CMV infections in pregnant women with CMV IgM positive serology.

The objective of this study was to evaluate a complete fully-automated CMV serology panel (CMV IgG, CMV IgM, CMV IgG avidity) that was developed on the Abbott ARCHITECT instrument.

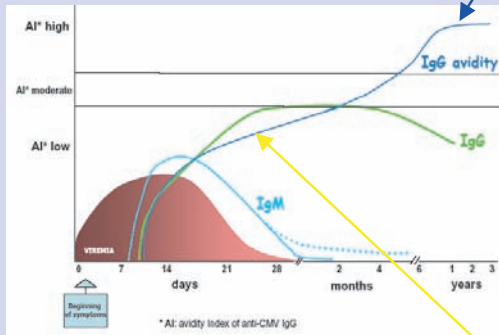
* In development – not available for sale

Methods and Procedures

Typical Time Course of CMV Seroconversion in Immunocompetent Individuals

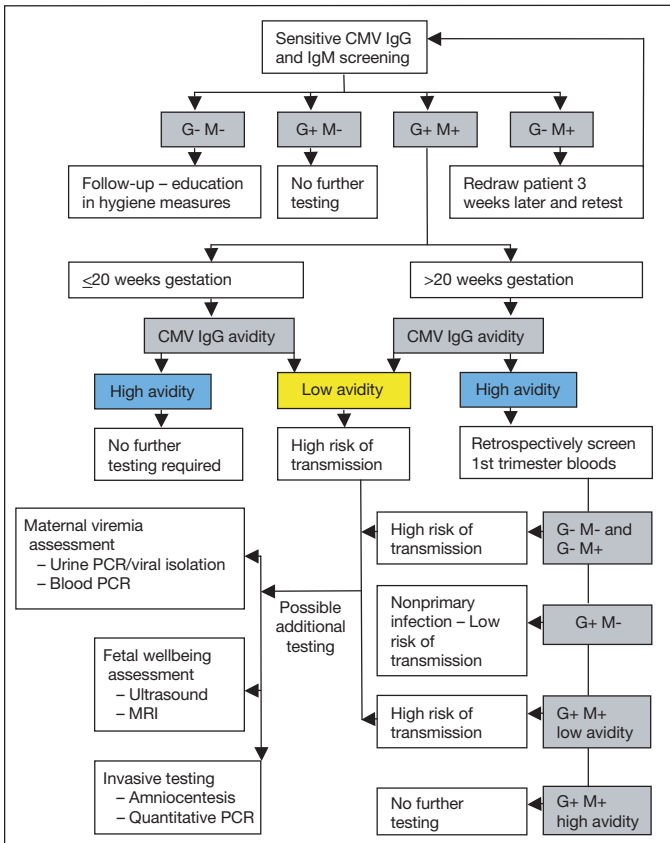
- IgG antibody of high avidity is detected in subjects with non-primary CMV infection.
- The maturation of antibody avidity over time can be used at the diagnostic level to discriminate between primary and non-primary infection.
- Avidity testing should be performed in pregnant women early in gestation (i.e., within the first trimester), as a high avidity result later in gestation cannot rule out a primary infection earlier in gestation when low avidity IgG may have been present.

Serology Characteristics

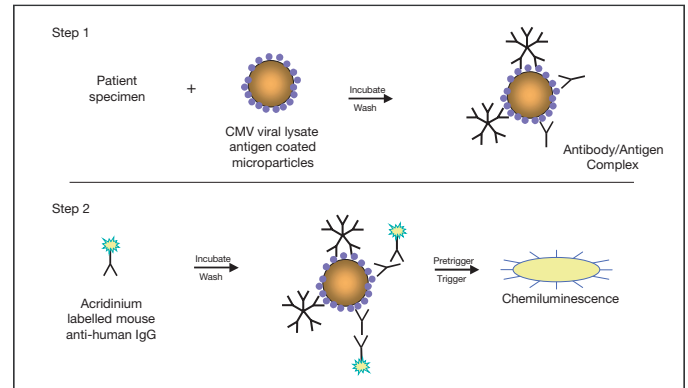


Low to moderate IgG avidity is a marker of CMV primary infection for 18 – 20 weeks after seroconversion.

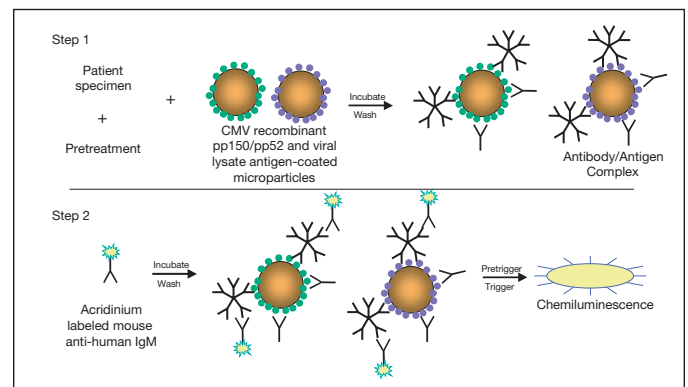
Proposed Diagnostic Algorithm for CMV Serology Screening in Pregnant Women¹



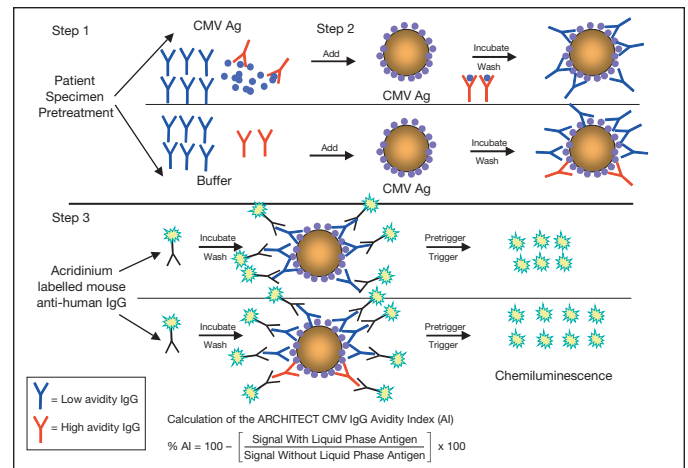
ARCHITECT CMV IgG Assay



ARCHITECT CMV IgM Assay



ARCHITECT CMV IgG Avidity Assay Using AVcomp Technology



¹Munro, S.C., et al. (2005) *J. Clin. Microbiol.* 43: 4713-4718.

Results

ARCHITECT CMV IgG Resolved Relative Sensitivity and Specificity

Samples from blood donors, pregnant women, transplant recipients, and hospitalized patients (n = 1,153)		Consensus After Resolution		
		Positive	Negative	Total
ARCHITECT CMV IgG	Positive	606	3	609
	Negative	0	544	544
	Total	606	547	1153

Resolved Relative Sensitivity: $606/606 = 100\%$ Resolved Relative Specificity: $544/547 = 99.5\%$

ARCHITECT CMV IgM Resolved Relative Specificity

Samples from blood donors and pregnant women (n = 706)		Consensus After Resolution		
		Positive	Negative	Total
ARCHITECT CMV IgM	Positive	3	7	10
	Negative	0	696	696
	Total	3	703	706

Resolved Relative Specificity: $696/703 = 99.0\%$

ARCHITECT and Radim CMV IgG Avidity Clinical Sensitivity

Samples within 4 months CMV post-seroconversion (n = 72)		Radim CMV IgG Avidity		
		Low	Equivocal	High
ARCHITECT CMV IgG Avidity	Low	59	8	3
	Grayzone	0	0	0
	High	0	1	1

ARCHITECT Clinical Sensitivity: $70/72 = 97.2\%^{**}$ Radim Clinical Sensitivity: $59/63 = 93.7\%^{**}$

^{**} Equivocal results excluded from calculation

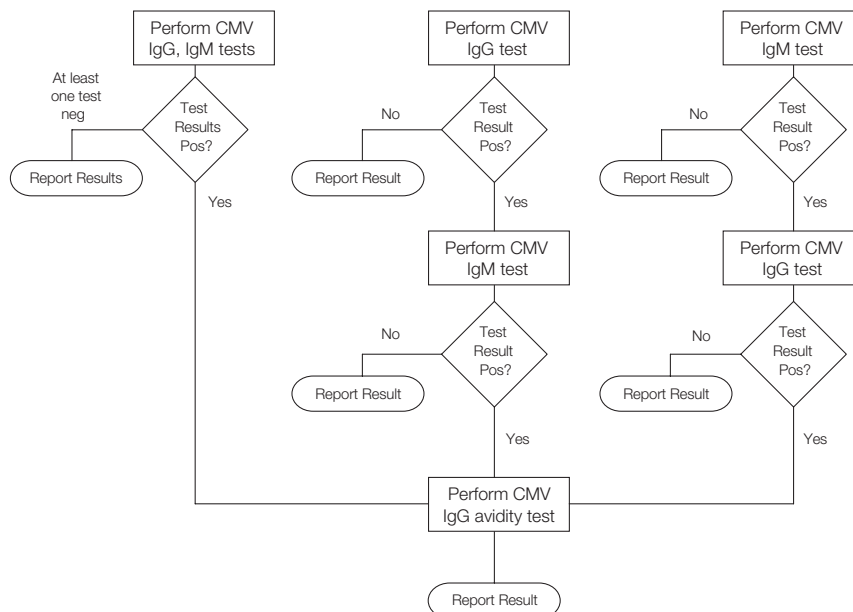
ARCHITECT and Radim CMV IgG Avidity Clinical Specificity

Samples from CMV immune blood donors and pregnant women, prescreened CMV IgG+ IgM- (n = 256)		Radim CMV IgG Avidity		
		Low	Equivocal	High
ARCHITECT CMV IgG Avidity	Low	1	0	0
	Grayzone	0	0	4
	High	1	0	250

ARCHITECT Clinical Specificity: $251/252 = 99.6\%^{**}$ Radim Clinical Specificity: $254/256 = 99.2\%^{**}$

^{**} Equivocal results excluded from calculation

Three Different Automated CMV Testing Algorithms Have Been Developed for the ARCHITECT Instrument



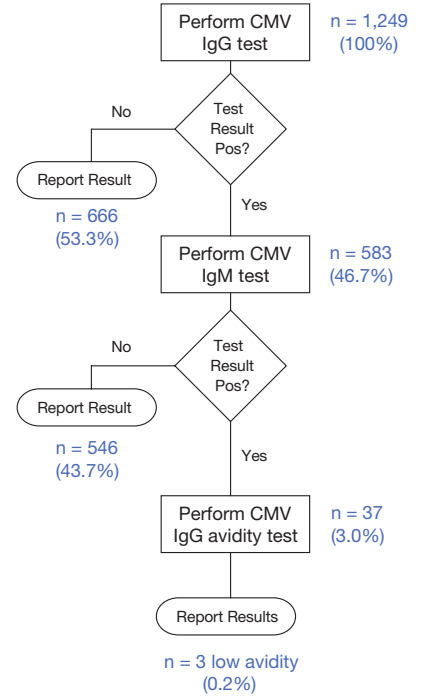
Results (cont.)

CMV Seroconversion Panels

Panel	Cutoff Grayzone to:		Architect CMV IgG	AxSYM CMV IgG	Vidas CMV IgG	Behring Enzygnost CMV IgG	Architect CMV IgM	IMx CMV IgM	AxSYM CMV IgM	Architect CMV IgG Avidity	RADIM CMV IgG Avidity
	Days	Months	6 AU/mL N/A	15 AU/mL N/A	6 AU/mL 4 AU/mL	0.200 0.100	1.00 0.85	0.60 0.50	0.50 0.40	50% 60%	35% 45%
	CMV IgG				CMV IgM			CMV IgG Avidity			
Profile RP003	1	0.0	7.3	25.7	<6	0.034	1.65	1.19	0.31	10%	n.t.
	4	0.1	22.7	54.9	6.0	0.087	4.29	3.09	1.20	2%	4%
	8	0.3	51.8	112.2	12.0	n.t.	4.42	3.66	2.64	8%	4%
	51	1.7	74.7	162.5	31.0	0.448	1.40	1.21	0.78	20%	29%
	55	1.8	75.6	167.3	33.0	0.466	1.32	0.97	0.80	22%	31%
	59	1.9	72.1	175.8	34.0	0.479	1.42	0.87	0.87	28%	31%
	65	2.1	67.7	166.7	31.0	0.444	1.20	0.67	0.80	28%	31%
	67	2.2	69.8	178.0	29.0	0.407	1.19	0.65	0.79	31%	26%
	72	2.4	64.5	170.7	25.0	0.371	1.12	0.56	0.75	35%	33%
	74	2.4	66.2	171.0	25.0	0.489	1.10	0.52	0.74	41%	34%
	79	2.6	62.9	156.4	23.0	0.503	1.11	0.53	0.81	39%	45%
	84	2.8	72.4	183.6	25.0	0.438	1.11	0.52	0.87	41%	41%
	88	2.9	78.3	185.8	26.0	0.319	1.13	0.52	1.00	41%	43%
	95	3.1	68.7	170.5	22.0	0.428	1.09	0.46	0.88	46%	47%
99	3.2	71.0	169.2	23.0	0.406	1.03	0.52	0.91	44%	39%	

Panel	Cutoff Grayzone to:		Architect CMV IgG	AxSYM CMV IgG	Vidas CMV IgG	Behring Enzygnost CMV IgG	Architect CMV IgM	IMx CMV IgM	Vidas CMV-M	AxSYM CMV IgM	Architect CMV IgG Avidity	RADIM CMV IgG Avidity	Vidas CMV IgG Avidity
	Days	Months	6 AU/mL N/A	15 AU/mL N/A	6 AU/mL 4 AU/mL	0.200 0.100	1.00 0.85	0.60 0.50	0.90 0.70	0.50 0.40	50% 60%	35% 45%	20% 80%
	CMV IgG				CMV IgM				CMV IgG Avidity				
Profile RP019	1	0.0	1.0	2.4	<6	0.028	0.20	0.14	n.t.	0.19			
	5	0.2	1.0	1.3	<6	0.021	0.18	0.11	n.t.	0.15			
	8	0.3	1.1	2.0	<6	0.018	0.00	0.13	n.t.	0.16			
	12	0.4	1.0	1.6	<6	0.023	0.25	0.11	n.t.	0.16			
	15	0.5	0.8	1.1	<6	0.044	0.19	0.11	n.t.	0.15			
	21	0.7	1.5	4.2	<6	0.018	0.28	0.21	n.t.	0.24			
	26	0.9	1.8	4.9	<6	0.057	0.35	0.21	0.19	0.19			
	29	1.0	3.2	10.4	<6	0.023	0.98	0.56	0.52	0.20			
	33	1.1	10.2	31.1	<6	0.075	4.09	2.37	1.62	0.63	9%	12%	
	36	1.2	16.7	35.9	<6	0.171	5.66	3.15	1.70	1.37	2%	11%	
	43	1.4	62.1	119.4	17.0	0.415	6.35	3.52	1.90	3.13	9%	6%	6%
	50	1.6	75.0	145.7	26.0	0.475	4.94	3.31	1.90	2.61	2%	4%	8%
	57	1.9	n.t.	167.4	40.0	0.670	4.35	2.72	1.90	2.31	7%	5%	15%
	68	2.2	102.2	188.4	50.0	0.784	3.14	2.22	1.70	1.65	17%	7%	26%
	75	2.5	100.7	195.1	51.0	0.747	2.80	1.88	1.40	1.36	23%	8%	30%
	82	2.7	102.1	187.1	n.t.	0.644	2.20	1.63	n.t.	1.22	26%	10%	32%
	86	2.8	103.3	186.6	n.t.	0.830	2.52	1.68	1.50	1.18	25%	11%	37%
	89	2.9	94.3	177.0	n.t.	0.877	2.31	1.44	1.40	1.05	26%	11%	42%
	96	3.1	97.1	176.5	n.t.	0.828	2.08	1.37	1.20	0.94	31%	13%	40%
	104	3.4	100.5	167.9	n.t.	0.797	1.99	1.31	1.20	0.87	33%	15%	45%
	109	3.6	86.8	172.4	n.t.	0.771	1.75	1.18	0.80	0.83	32%	17%	45%
	113	3.7	94.4	189.5	n.t.	0.573	2.12	1.28	1.30	0.91	32%	16%	47%
	116	3.8	105.7	199.1	n.t.	0.680	2.02	1.32	1.30	0.94	33%	17%	47%
	121	4.0	95.5	194.3	n.t.	0.541	1.74	1.10	n.t.	0.78	35%	18%	49%
124	4.1	100.2	189.3	n.t.	0.571	1.93	1.08	0.73	0.80	37%	20%	51%	

Sample Testing Using One of the CMV Algorithms Developed for the ARCHITECT Instrument



Automation of CMV Testing Algorithms

- For each sample the desired retest rule may be chosen individually and ordered like a single assay. The decision criteria are user editable and may be adapted to specific user needs.
- CMV IgG Avidity is tested in combination with CMV IgG, because the CMV IgG result is the basis for the automated dilution of samples for CMV IgG avidity testing.
- Automatic reflex testing will be available on the ARCHITECT *i*2000_{SR} and *i*1000* instruments.
- For a sample that has to be tested in all 3 assays the maximal sample volume required is 250 µL.

Conclusion

- The ARCHITECT CMV panel includes three assays: CMV IgG, IgM and IgG avidity
- The performance of the ARCHITECT CMV IgG avidity assay using “AVIcomp technology” was superior to the Radim CMV IgG avidity assay
- The ARCHITECT instrument offers complete automation of the CMV panel including avidity with the reflex test option available on the *i*2000_{SR} and *i*1000* instruments.
- Three automated CMV testing algorithms have been developed for the CMV Panel on the ARCHITECT instrument that can be edited by the user, thus allowing adaptation of the retest rules to specific requirements of the laboratory.
- Inclusion of the CMV IgG avidity assay in the CMV panel allows laboratories to perform the avidity test “in-house”, thereby reducing laboratory cost and turnaround time.