New Developments in Congenital Testing and Implications for Patient Management

Gregory Maine, Ph.D.
Global Scientific Affairs Manager
Associate Research Fellow

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Overview

- Review CMV and Toxo Infection
- Review Diagnostic Tests and Algorithms
- Review Design and Performance of the New Abbott ARCHITECT Congenital Panel

Acquisition of Primary CMV or Toxo Infection During Gestation

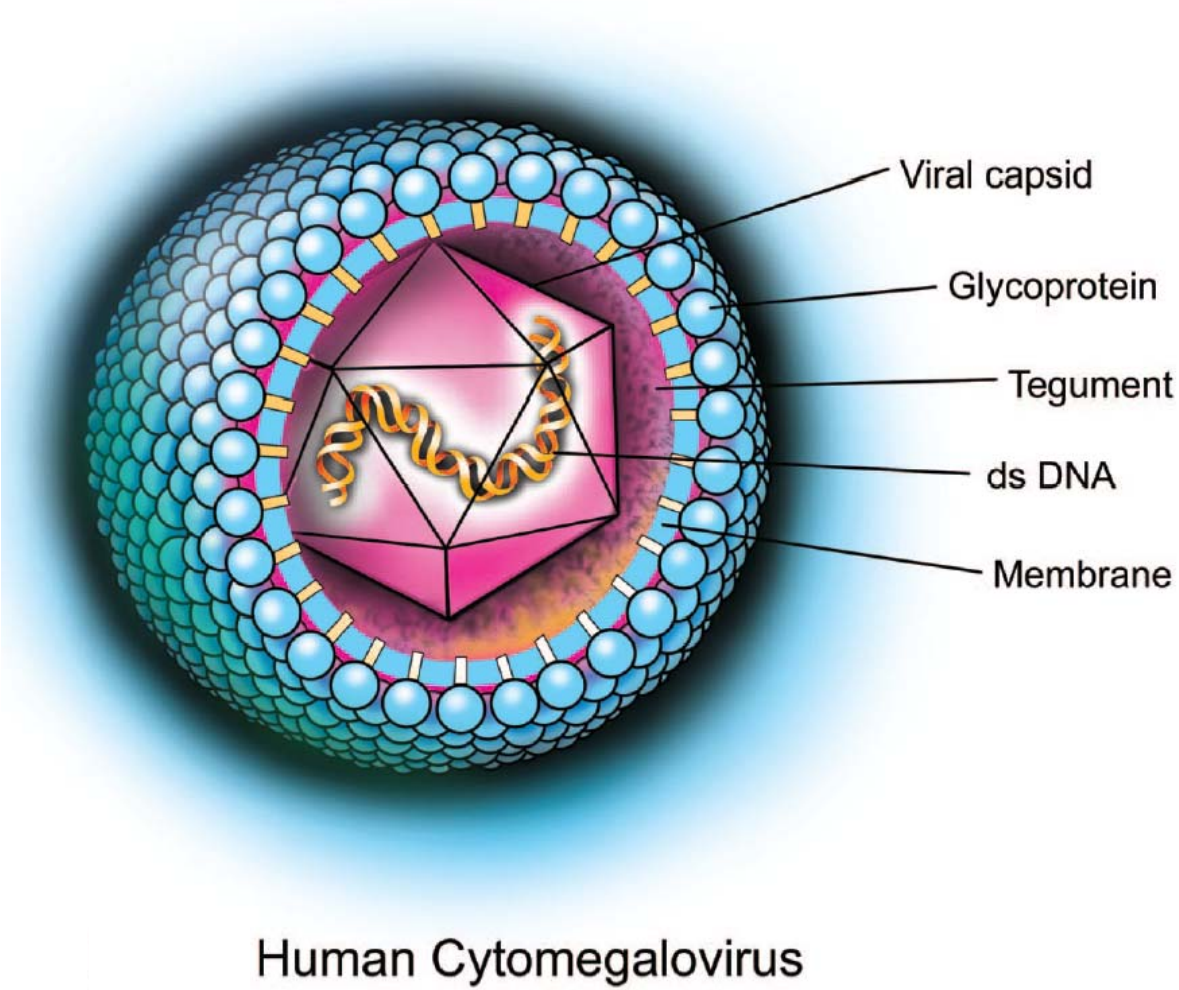
Though not serious for pregnant women, intrauterine transmission of infection causes the most fetal damage when acquired in the first trimester

- CMV: Most common Congenital infection, there is no vaccine, prevention must be stressed, pre-pregnancy serology, experimental maternal treatment available*

- Toxo: There is no vaccine, prevention must be stressed, pre-pregnancy serology, maternal treatment is available

*Maine, GT et al. (2001) Expert Rev. Mol. Diagn. 1:19-29

CMV



CMV Serology is Performed by...

Where?	Blood Bank	Private or Hospital Lab
Why?	Minimize risk to transmit CMV to immunocompromised patients	Identify primary CMV infections in pregnant women that are at high risk to transmit CMV to the unborn child.
What?	**CMV IgG tested**	**CMV IgG tested** / **CMV IgM tested**
		CMV IgG Avidity can be tested

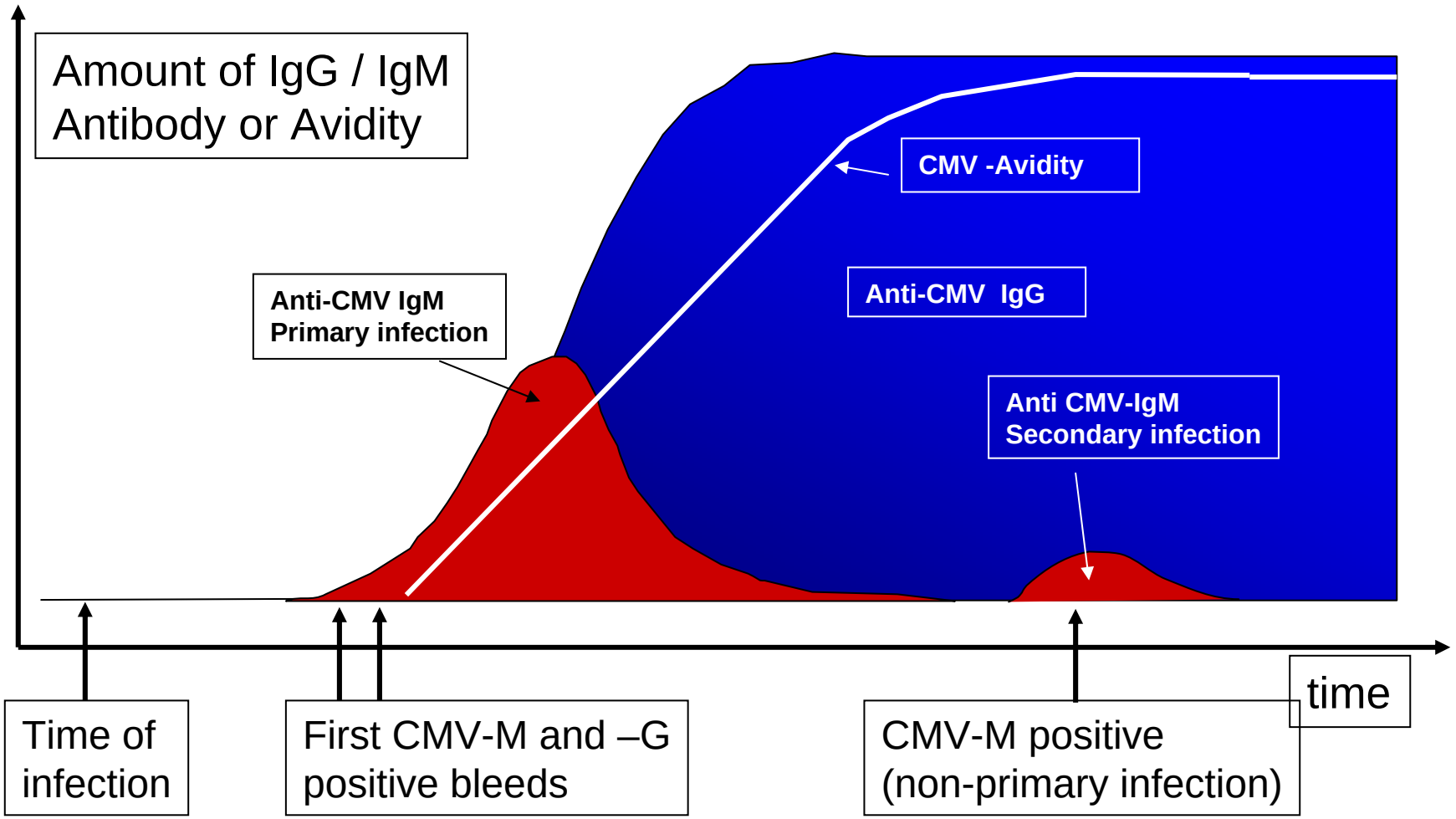
CMV-IgG, IgM and IgG Avidity assays are essential for the reliable identification of primary CMV infections.

CMV Infection

- CMV is the most common congenital infection*
- Congenital CMV infection occurs in approximately 1% of all live births
- Primary intrauterine CMV infections rank second behind Down's syndrome as a cause of mental retardation

*Maine, GT et al. (2001) Expert Rev. Mol. Diagn. 1:19-29

Schematic Overview of CMV Infection



Laboratory Methods for the Diagnosis of CMV Infection

- CMV Culture
- CMV Serology (IgM, IgG, IgG Avidity)
- Application of CMV PCR in prenatal (AF) and postnatal diagnosis

ARCHITECT Congenital Assays

ARCHITECT: CMV IgG, IgM, IgG Avidity

ARCHITECT: Toxo IgG, IgM, IgG Avidity

ARCHITECT: Rubella IgG, IgM

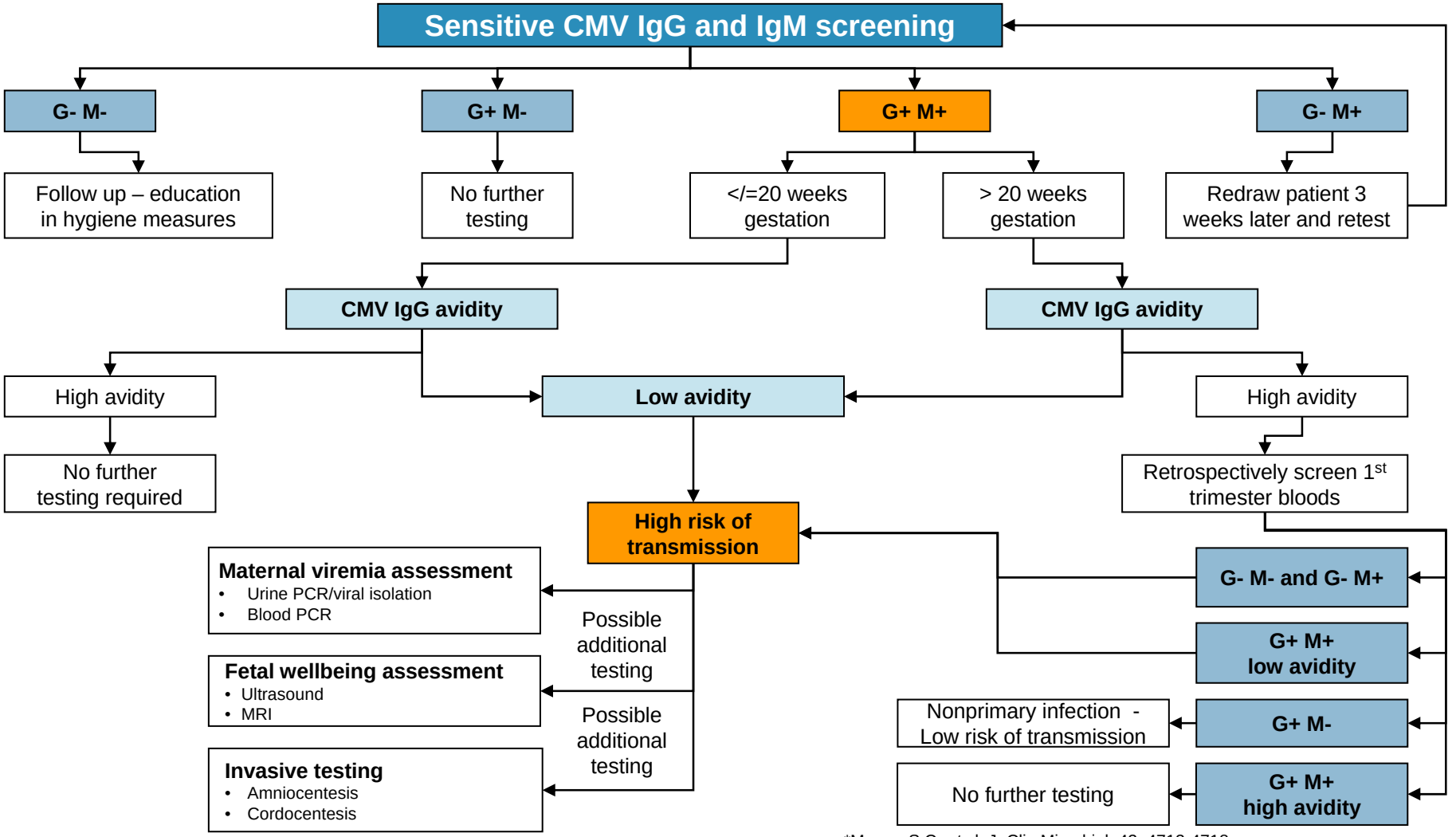
***i*2000**

***i*2000$_{SR}$**

***i*1000$_{SR}$**

Fully automated, high throughput system with integrated avidity assays

Diagnostic Algorithm for CMV Serology Screening in Pregnant Women*



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

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Validity of Diagnostic Strategy: Incidence of Congenital CMV Infection

Longitudinal study at the University of Bologna: 1994-2005*

Cohort 2,477 pregnant women

Table 2
Results of diagnosis of maternal CMV infection by serologic tests and pregnancy outcomes

Maternal infection	Total (%)	IgM blot positive	Low/mod AI	High AI	Fetuses/newborns examined	Congenital infection (%)
Not active	1367 (55.1)	0	0	1367	977	1 (0.1)
Primary true IgM plus low/mod avidity	514 (20.8)	514	514	0	484	121 (25.0)
Primary seroconversion	183 (7.4)	183	183	0	175	53 (30.3)
Non-primary	336 (13.6)	336	0	336	292	6 (2.0)
Undefined	77 (3.1)	77	mod 65	12	64	3 (4.7)
Total	2477	1110	762	1715	1992	184

AI: avidity-IgG index; mod: moderate.

Incidence of congenital CMV infection was similar when primary CMV infection was detected by IgM+/low/moderate avidity or seroconversion

*Lazzarotto et al. (2008) J. Clin. Virol. 41:192

Validity of Diagnostic Strategy: Seroconversion Study in Pregnant Women

Evaluation of the Abbott ARCHITECT CMV Panel in Pregnant Women with Primary CMV Infection

G.T. Maine[1], R. N. Stricker[2], R.T. Stricker[2]

[1]Abbott Diagnostics, Abbott Park, IL, [2]Dianalabs, Geneva, Switzerland

2nd Annual Conference on Congenital CMV Infection and Disease, Atlanta, Georgia • November 5-7, 2008

Validity of Diagnostic Strategy: Seroconversion Study in Pregnant Women

Methods and Procedures

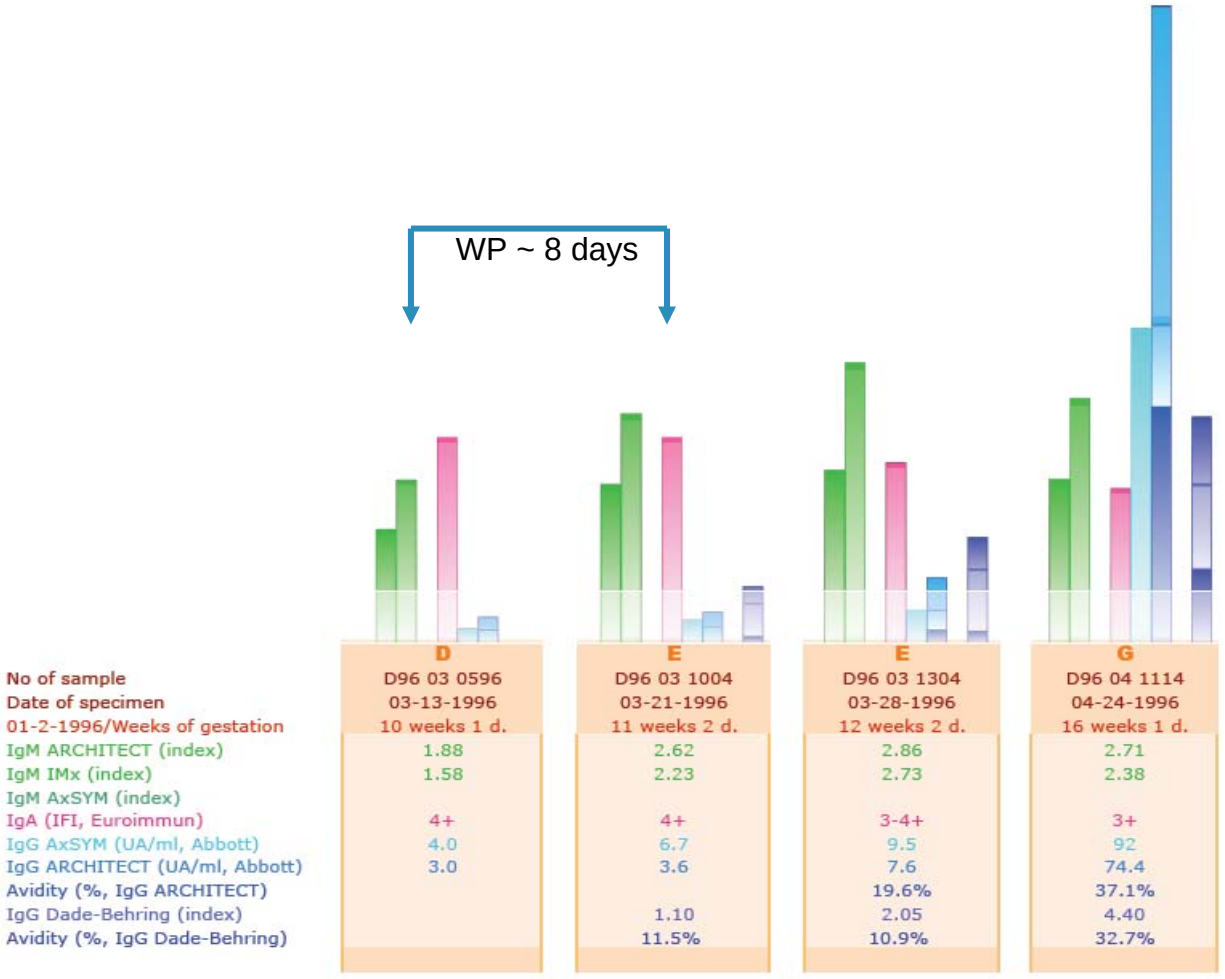
- Archived samples (n = 136) from 1996-2006 were selected from 31 pregnant women with documented recent CMV seroconversion.
- In this patient population (n = 31) the mean days from last seronegative bleed to the first seropositive bleed was 43 days, 21 days from midpoint.

Validity of Diagnostic Strategy: Seroconversion Study in Pregnant Women

Key Result

Detection of CMV-specific IgM before CMV IgG occurred in 6/31 patients (19%) with a window period of approximately 8-13 days

CMV Seroconversion Patient No. 879



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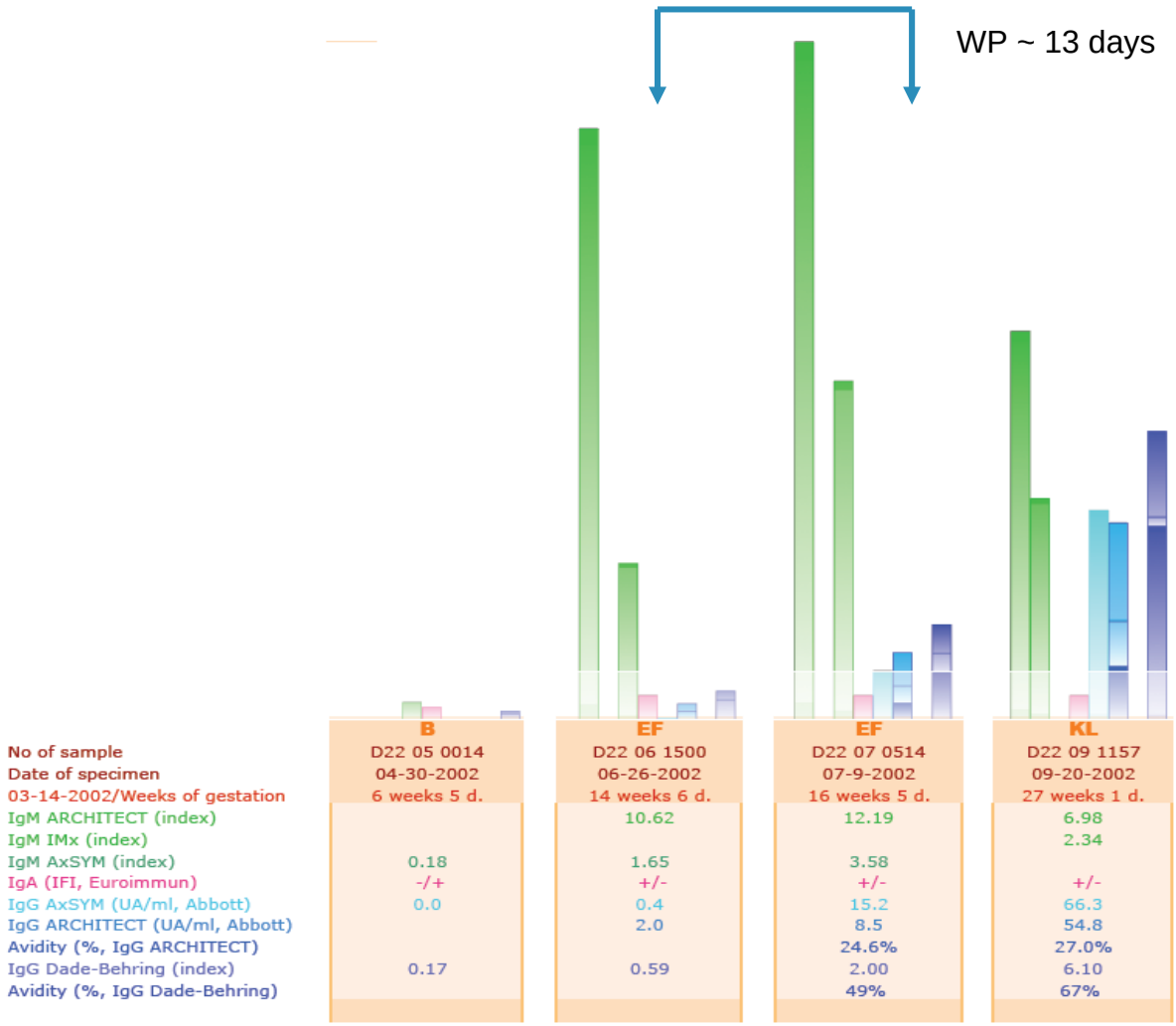
CMV Seroconversion Patient No. 1951



	B	EF	EF	KL
No of sample	D22 05 0014	D22 06 1500	D22 07 0514	D22 09 1157
Date of specimen	04-30-2002	06-26-2002	07-9-2002	09-20-2002
03-14-2002/Weeks of gestation	6 weeks 5 d.	14 weeks 6 d.	16 weeks 5 d.	27 weeks 1 d.
IgM ARCHITECT (index)		10.62	12.19	6.98
IgM IMx (index)				2.34
IgM AxSYM (index)	0.18	1.65	3.58	
IgA (IFI, Euroimmun)	-/+	+/-	+/-	+/-
IgG AxSYM (UA/ml, Abbott)	0.0	0.4	15.2	66.3
IgG ARCHITECT (UA/ml, Abbott)		2.0	8.5	54.8
Avidity (%, IgG ARCHITECT)			24.6%	27.0%
IgG Dade-Behring (index)	0.17	0.59	2.00	6.10
Avidity (%, IgG Dade-Behring)			49%	67%

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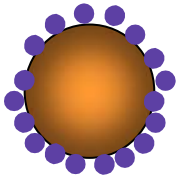
ARCHITECT CMV IgG Assay

Step 1

Patient specimen

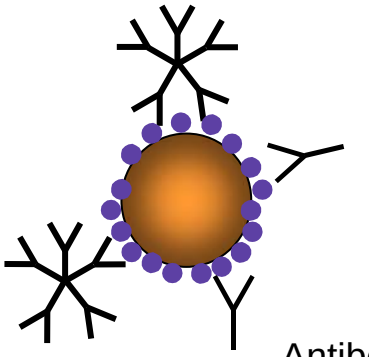
+



CMV viral lysate antigen coated microparticles

Incubate

Wash



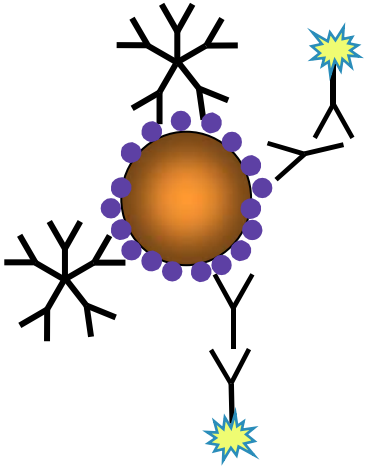
Antibody/Antigen Complex

Step 2

Acridinium labelled mouse anti-human IgG

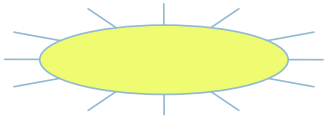
Incubate

Wash



Pretrigger

Trigger



Chemiluminescence

ARCHITECT CMV IgM Assay

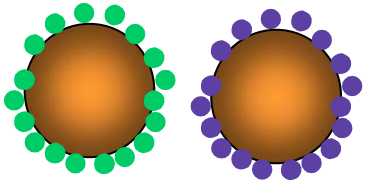
Step 1

Patient specimen

+

Pretreatment

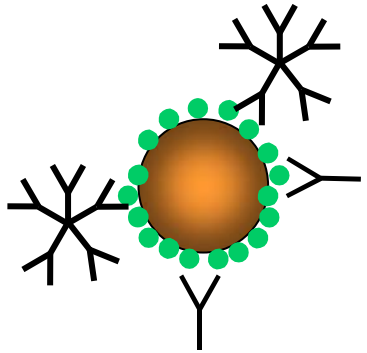
+

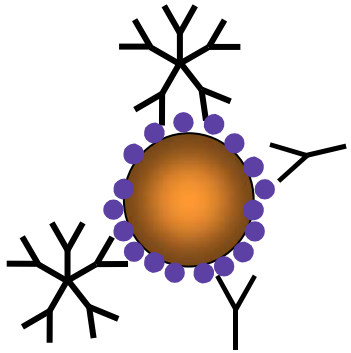


CMV pp150/pp52 and viral lysate antigen coated microparticles

Incubate

Wash





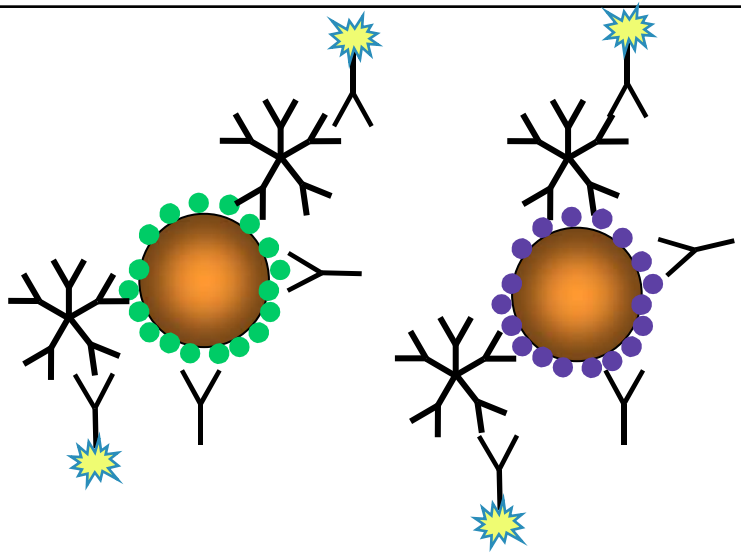
Antibody/Antigen Complex

Step 2

Acridinium labelled mouse anti-human IgM

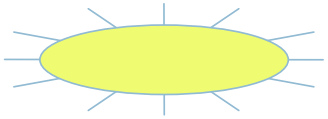
Incubate

Wash



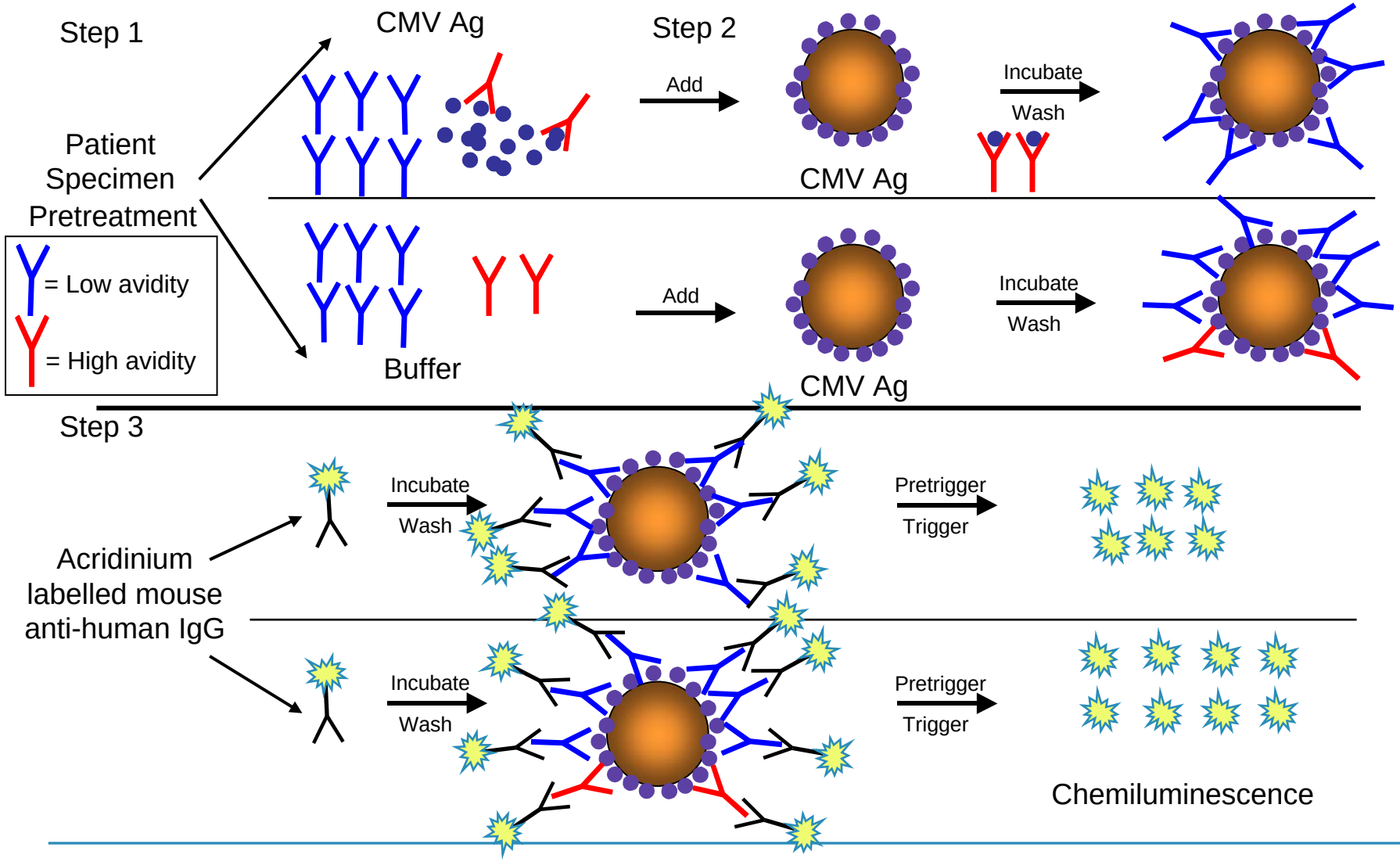
Pretrigger

Trigger



Chemiluminescence

ARCHITECT CMV IgG Avidity Assay using AVIcomp Technology



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ARCHITECT CMV IgG Resolved Sensitivity and Specificity

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

Resolved Sensitivity: 606/606 = 100%

Resolved Specificity: 544/547 = 99.5%

ARCHITECT CMV IgM Agreement on a Pregnant Women Population

	AxSYM CMV-M			Vidas CMV-M		
ARCHITECT	NEG	GZ	POS	NEG	GZ	POS
NEG	288	2	12	269	4	4
GZ	2	0	1	2	0	1
POS	2	0	3	4	0	1
Agreement	93.9%			94.7%		

ARCHITECT and Radim CMV IgG Avidity Clinical Sensitivity

N=72		Radim Avidity		
		Low	gz	high
Architect CMV IgG Avidity*	low	59	8	3
	gz	0	0	0
	high	0	1	1

Clinical Sensitivity on all seroconversion bleeds < 4 month:

ARCHITECT: 97.2% (70/72)

Radim: 93.7% (59/63)

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
ARCH CMV IgG Avidity	Low	1	0	0
	Equivocal	0	0	4
	High	1	0	250

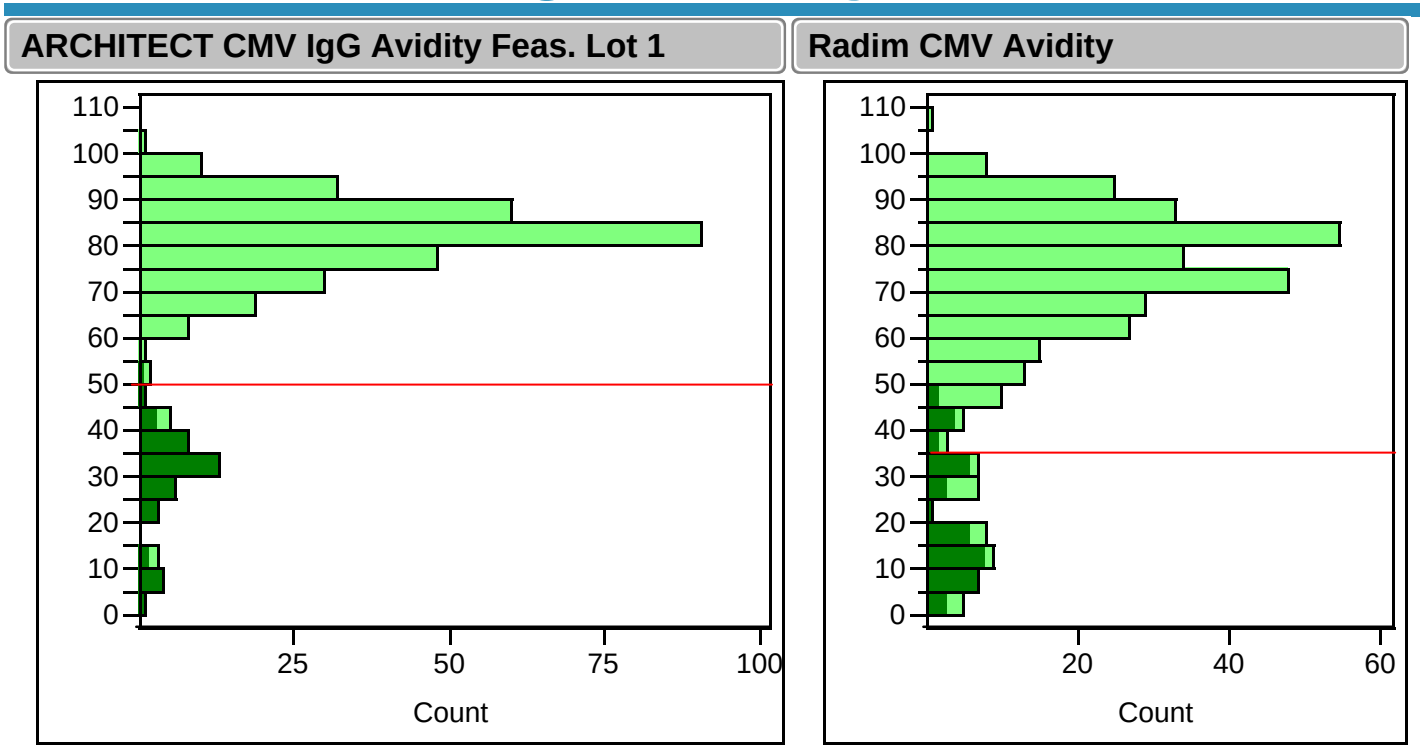
ARCHITECT Clinical Specificity: 251/252 = 99.6%*

Radim Clinical Specificity: 254/256 = 99.2%*

*Equivocal results excluded from calculation

*In development

Population Distribution on ARCHITECT and RADIM CMV IgG avidity



Samples pre-characterized as high avidity CMV G pos/ M neg

Samples pre-characterized as low avidity CMV seroconverter

ARCHITECT CMV Performance Data
Seroconversion Panel

			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
	Cutoff		**6 AU/ml**	**15 AU/ml**	**6 AU/mL**	**0.200**	**1.00**	**0.60**	**0.90**	**0.50**	**50%**	**35%**	**20%**
	Grayzone to:		**N/A**	**N/A**	**4 AU/mL**	**0.100**	**0.85**	**0.50**	**0.70**	**0.40**	**60%**	**45%**	**80%**
Panel	Days	Months	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	not detectable because CMV-IgG negative	not detectable because CMV-IgG negative	not detectable because CMV-IgG negative or too low
	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%

ARCHITECT CMV Performance Data
Seroconversion Panel

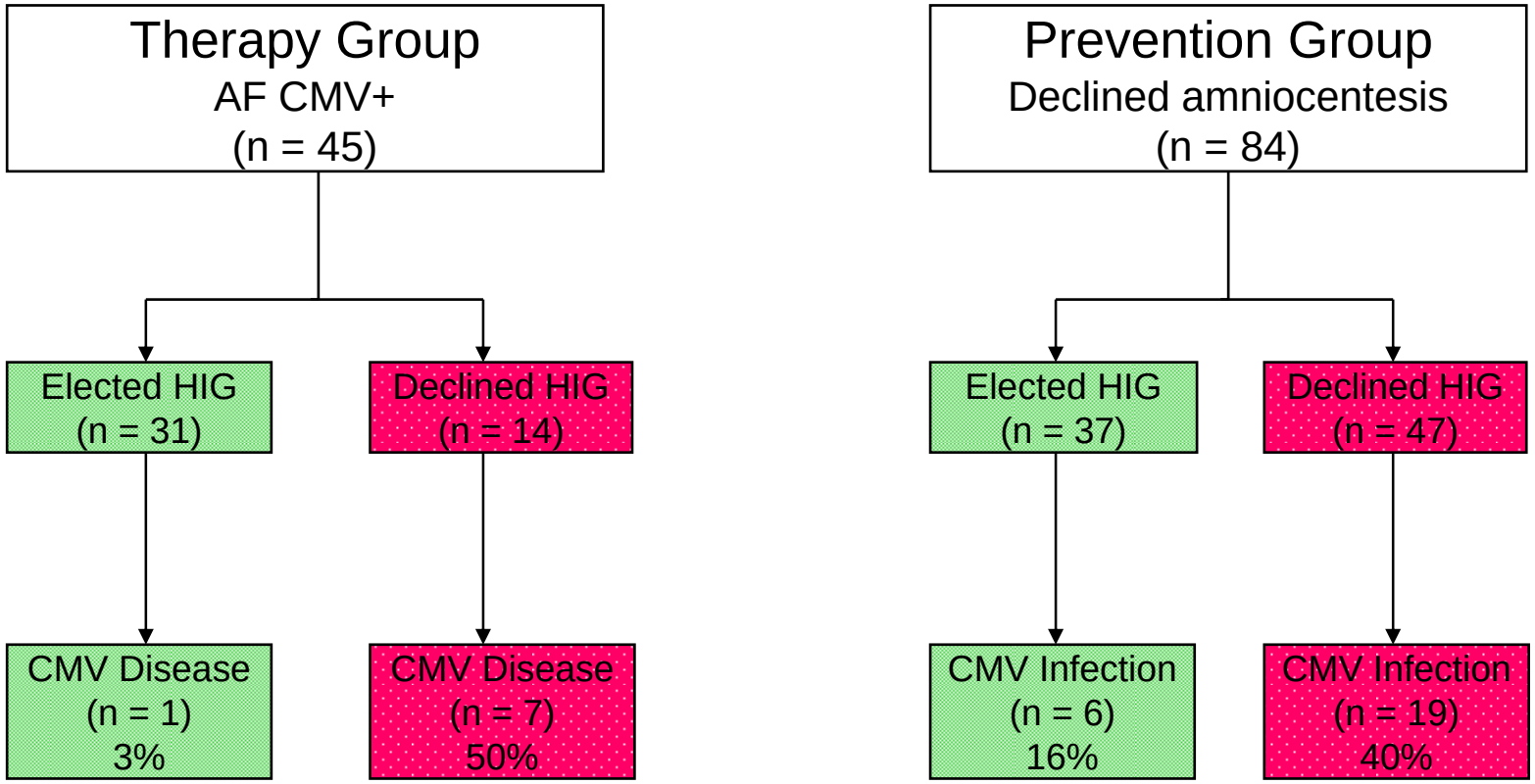
	CMV-G assays		CMV-M assays			CMV Avidity assays		
Months after first negative bleed	AXSYM CMV G cutoff 15 AU/ml	ARCH CMV G* cutoff 6 AU/ml	IMx CMV IgM (index 0.50-0.60)	AxSYM CMV IgM (index 0.40-0.50)	ARCH CMVM* (S/CO)	Avidity (IgG Dade-Behring)	%Avidity (RADIM)	%Avidity (ARCH*)
0.0	0.2	0		0.1	0.13	nt		
2.7	84	46		1.16	1.93	38%	28%	43%
3.1	117	54.2	1.61	1.01	1.69	41%	31%	46%
7.6	200	144.9	0.67		0.56	75%	69%	76%
8.3	400	154.2	0.37		0.46	83%	78%	79%

Maternal Treatment for CMV Infection

Passive immunization with hyperimmune globulin

Nigro *et al.* (2005) *New England Journal of Medicine* 353:1350-1362

Uncontrolled Study: “Passive immunization during pregnancy for congenital cytomegalovirus infection”*



P <0.001

P = 0.04

* Nigro *et al.* (2005) *New England Journal of Medicine* 353:1350-1362

BioTest and Abbott Cooperate on Clinical Trial for the Expansion of the Indication for Cytotect®

- Trial with more than 20,000 participants for the approval of the immunoglobulin in the indication of maternal primary CMV infection
- Abbott Diagnostics provides CMV tests and instrumentation
- Goal to confirm the results of the Italian study

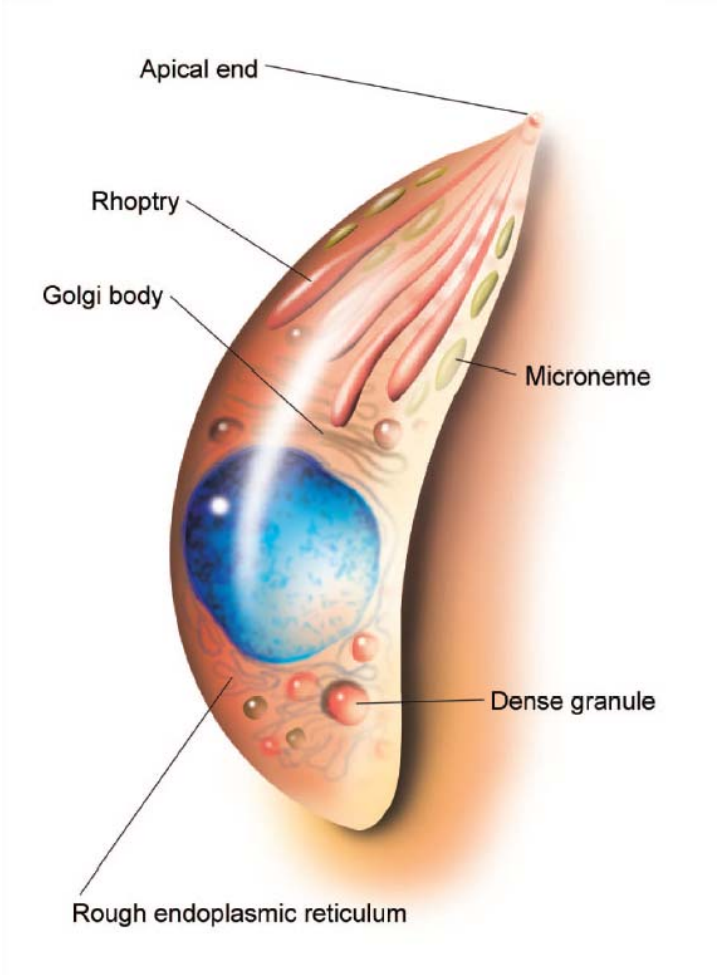
Treatment

- HIG treatment for primary maternal CMV infection is experimental and an alternative to pregnancy termination
- GCV treatment of infants with disease symptoms

Prevention

- Use of gloves when changing diapers
- Good hand washing after diaper change
- Avoidance of kissing on the mouth of young children
- Sanitize the toys of children, and be aware when you touch unsanitized toys

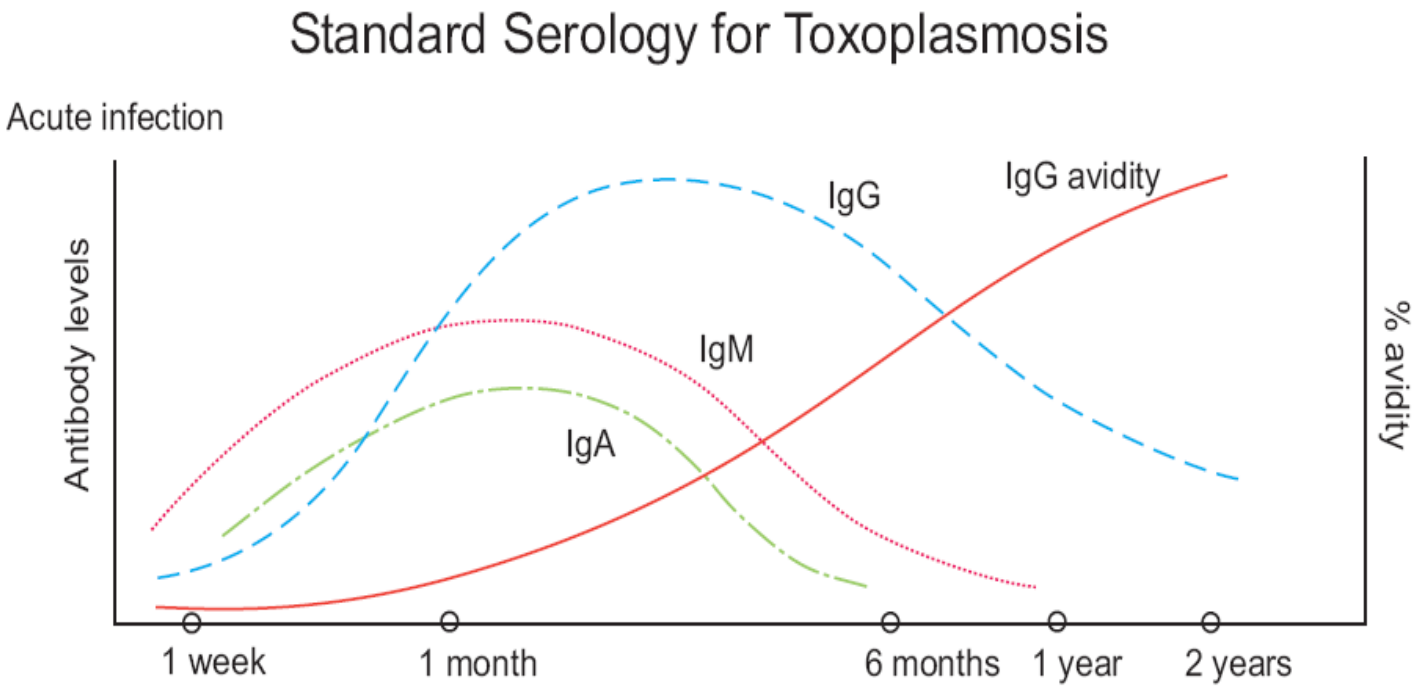
Toxoplasma gondii



Toxo Infection

- *T. gondii* can cause devastating disease in the fetus and newborn yet remain unrecognized in women who acquired the infection in pregnancy
- Congenital Toxo infection varies widely with geographic location occurs in approximately 0.1-0.01% of all live births

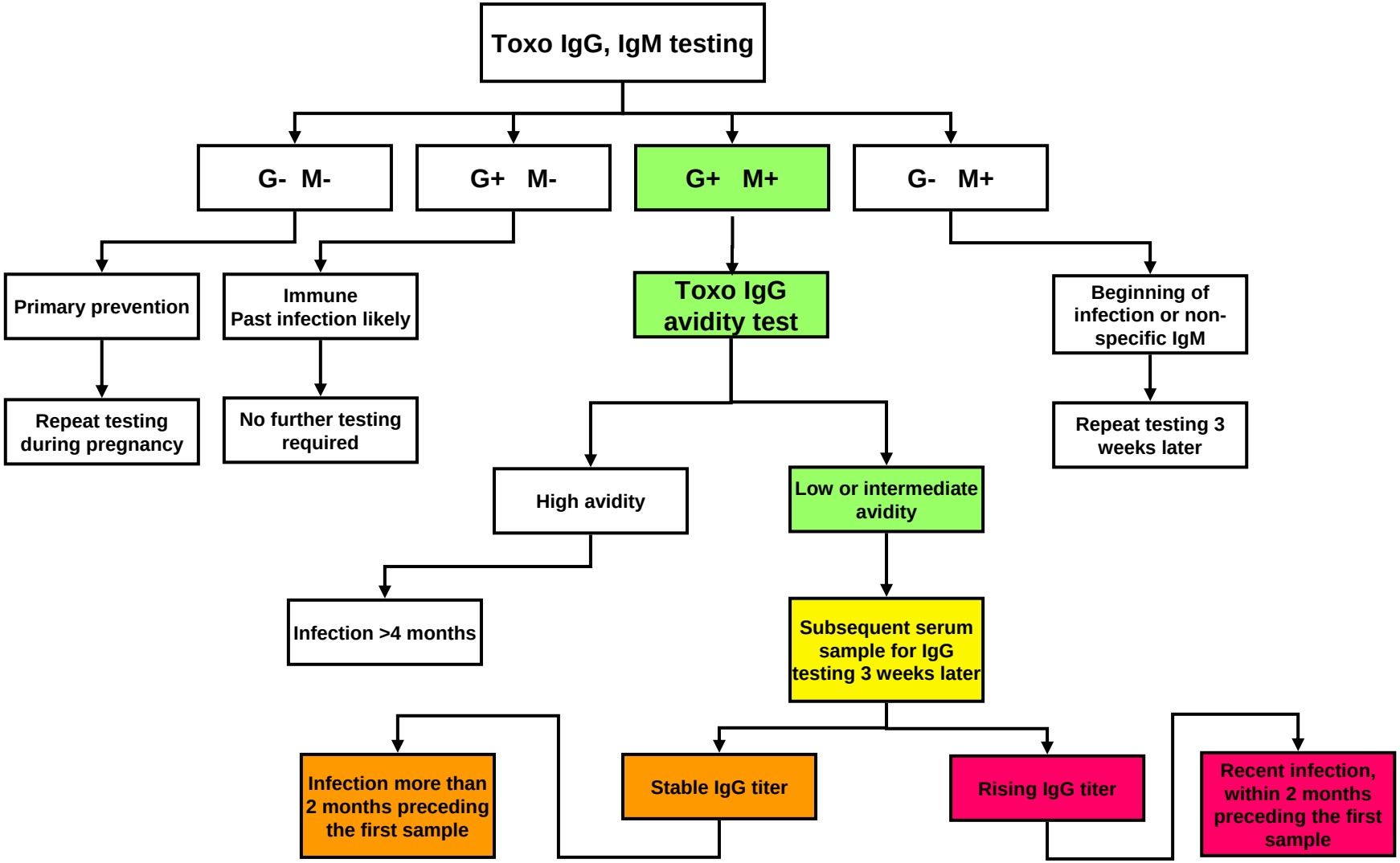
Schematic Overview of Toxo Infection



Laboratory Methods for the Diagnosis of Toxo Infection

- Mouse Inoculation and Cell Culture
- Biopsy
- Toxo Serology (IgM, IgG, IgG Avidity)
- Application of Toxo PCR in prenatal (AF) and postnatal diagnosis

Toxo Diagnostic Algorithm for Pregnant Women

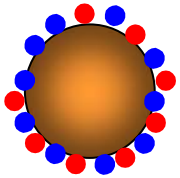


ARCHITECT Toxo IgG Assay*

Step 1

Patient specimen

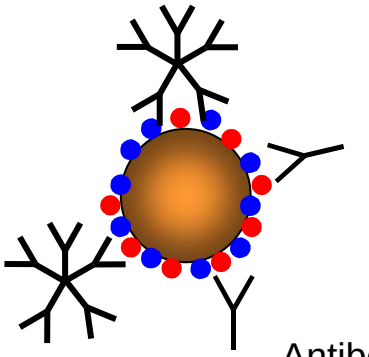
+



Toxo P30 (SAG1)
P35 (GRA8) antigen
coated microparticles

Incubate

Wash



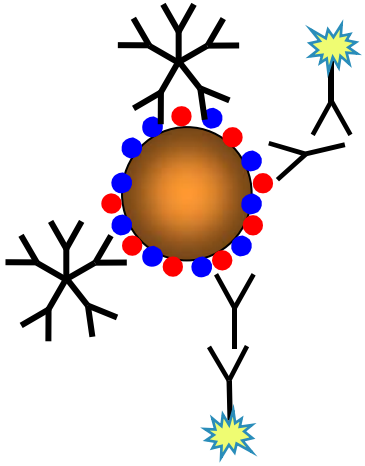
Antibody/Antigen Complex

Step 2

Acridinium labelled mouse anti-human IgG

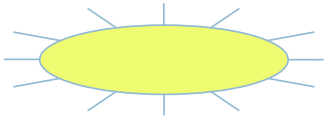
Incubate

Wash



Pretrigger

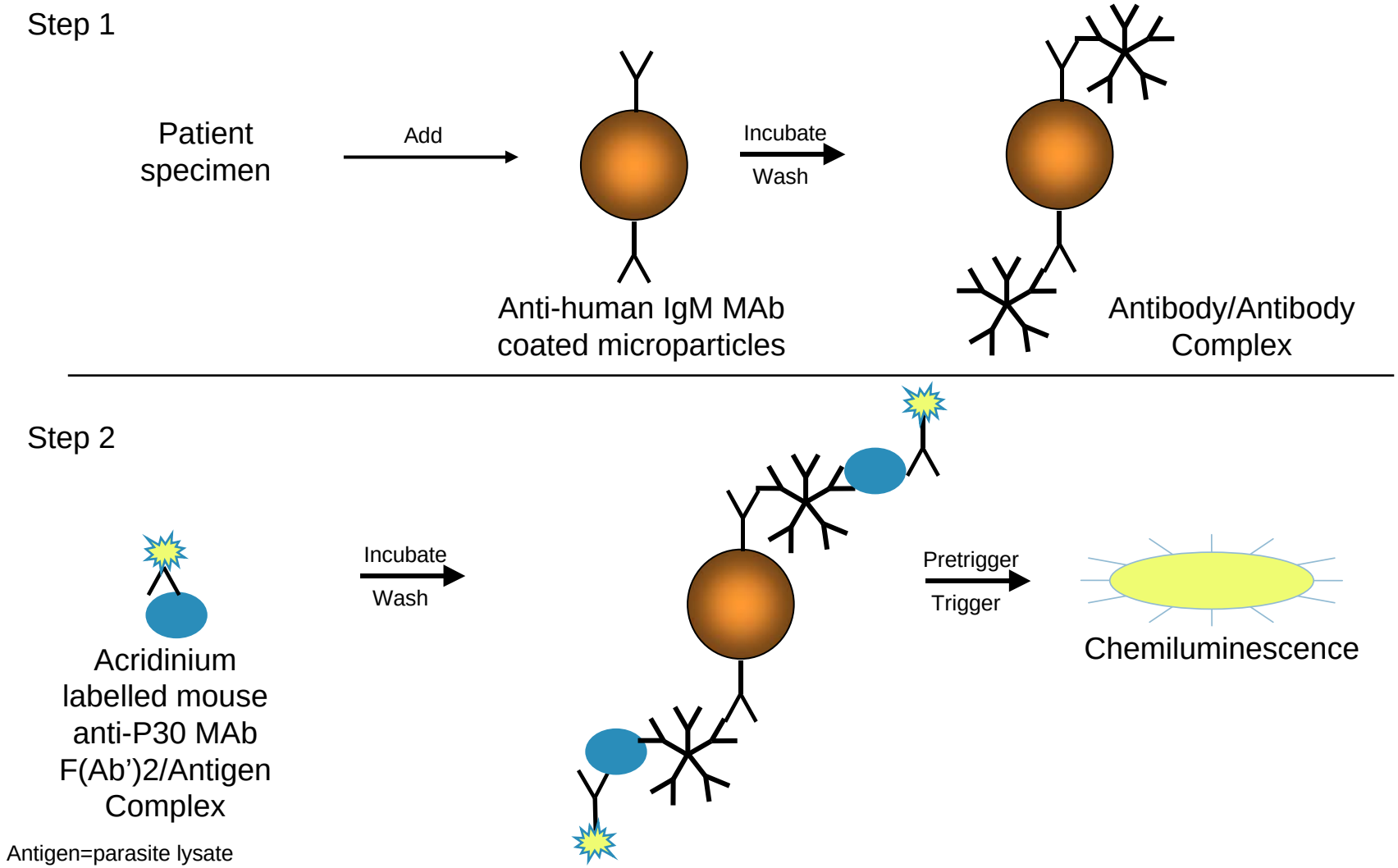
Trigger



Chemiluminescence

*Standardization: WHO approved 1st Int'l Standard Anti-Toxoplasma IgG, Human 2003, 20 IU/ampoule

ARCHITECT Toxo IgM Assay



Antigen=parasite lysate

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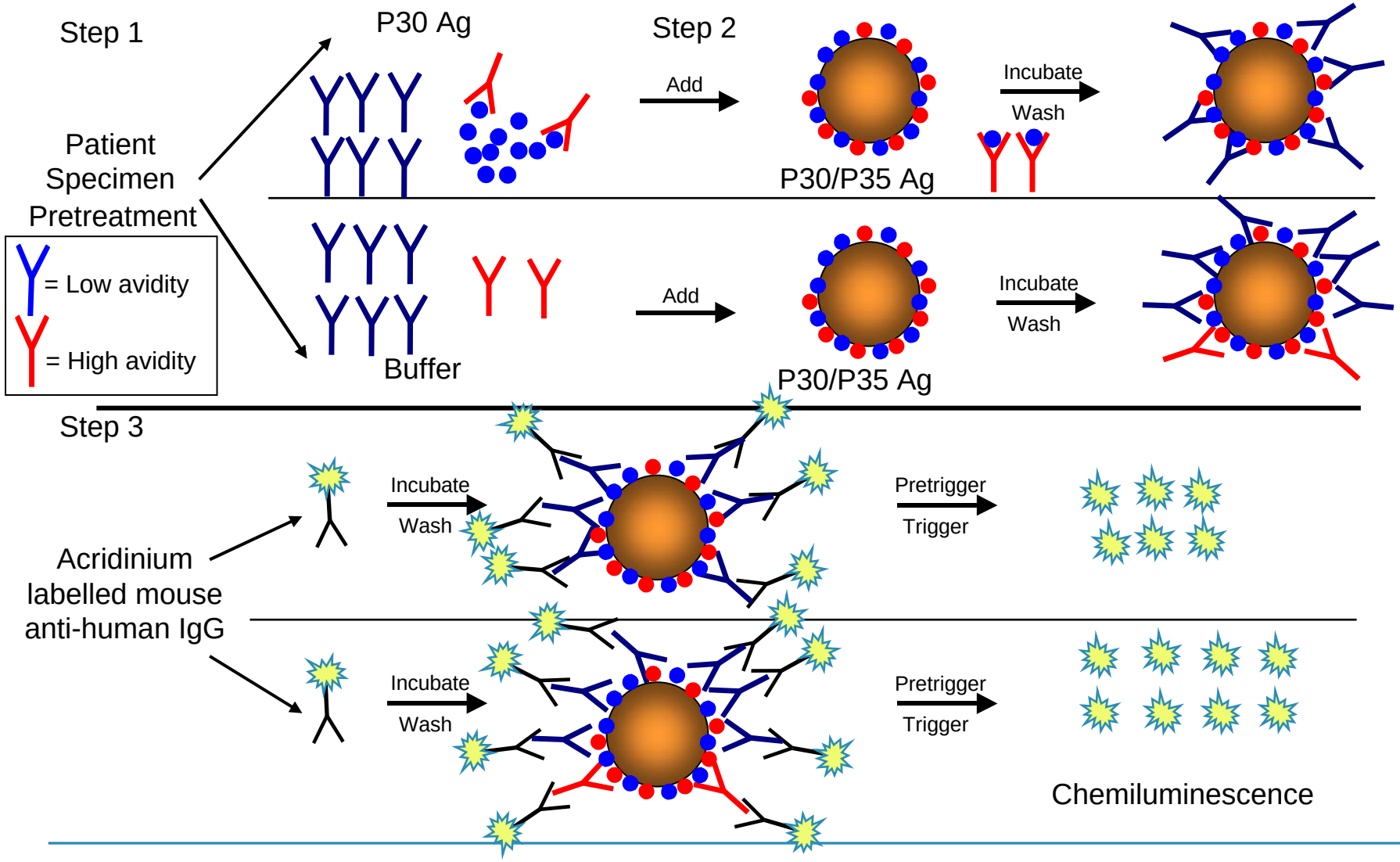
ARCHITECT Toxo IgG Avidity Assay using AVIcomp Technology



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ARCHITECT Toxo IgG Resolved Sensitivity and Specificity

Samples from blood donors and pregnant women (n = 820)		Consensus After Resolution		
		Positive	Negative	Total
ARCH Toxo IgG	Positive	403	3	406
	Negative	1	413	414
	Total	404	416	820

Resolved Sensitivity: 403/404 = 99.8%

Resolved Specificity: 413/416 = 99.3%

ARCHITECT Toxo IgG Performance Data
Seroconversion Panels

dd/mm/yy	months after first bleed	DT	HS	Isaga IgM	AxSYM Toxo G IU/ml	AxSYM Toxo M	ARCH Toxo IgG IU/ml
10/9/2003	0.0	<2	<1	0	1.2	0.325	0.5
11/10/2003	1.1	5	1	12	**3.5**	3.784	**1.3**
2/12/2004	4.2	200	32	12	108.5	2.255	32.5
7/31/2003	0.0	<2	<1	0	0.3	0.08	0.4
8/28/2003	0.9	2	<1	12	0.5	0.891	0.6
9/4/2003	1.2	10	4	12	5.1	2.647	2.6
1/18/2003	0.0	2	<1	11	**1.3**	0.93	**2.0**
2/20/2003	1.1	400	32	12	122	2.48	44.6
7/12/2003	5.8	400	32	12	84.9	1.023	45.4
3/15/2003	0.0	<2	<1	0	0.7	0.169	0.2
4/8/2003	0.8	5	2	9	7.6	2.782	10.0
4/30/2003	1.5	100	64	12	156.8	2.798	64.0
9/24/2003	6.4	80	32	12	35.9	0.859	14.1

ARCHITECT Toxo IgM Resolved Specificity

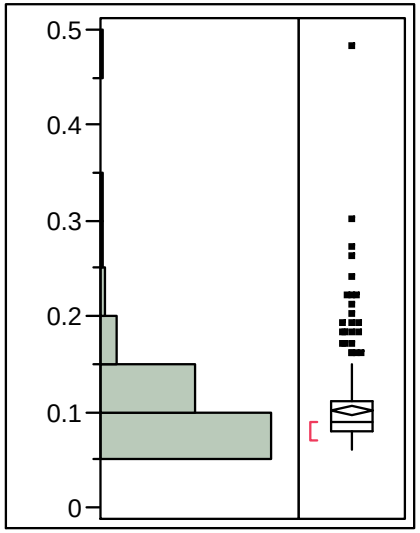
Samples pregnant women and random individuals (blood donors and diagnostic specimen) (n = 992)		Consensus After Resolution		
		Positive	Negative	Total
ARCH Toxo IgM	Positive	3	3	6
	Negative	0	986	986
	Total	3	989	992

Resolved Specificity: 986/989 = 99.7%

ARCHITECT and AxSYM Toxo IgM: Random Routine Specimen Testing at Dianalab

Distributions

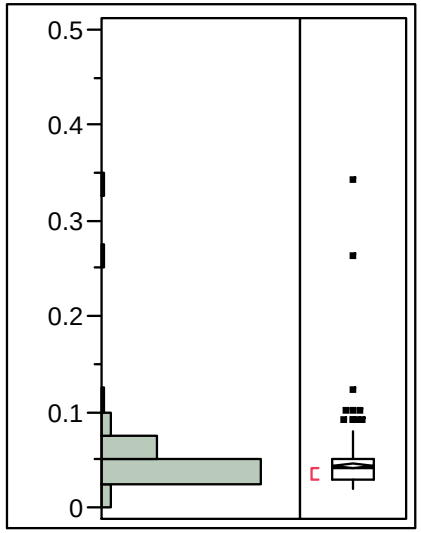
AxSYM Toxo IgM



Moments

Mean	0.1011438
Std Dev	0.0420421
Std Err Mean	0.0024034
upper 95% Mean	0.1058731
lower 95% Mean	0.0964145
N	306

Architect Toxo IgM



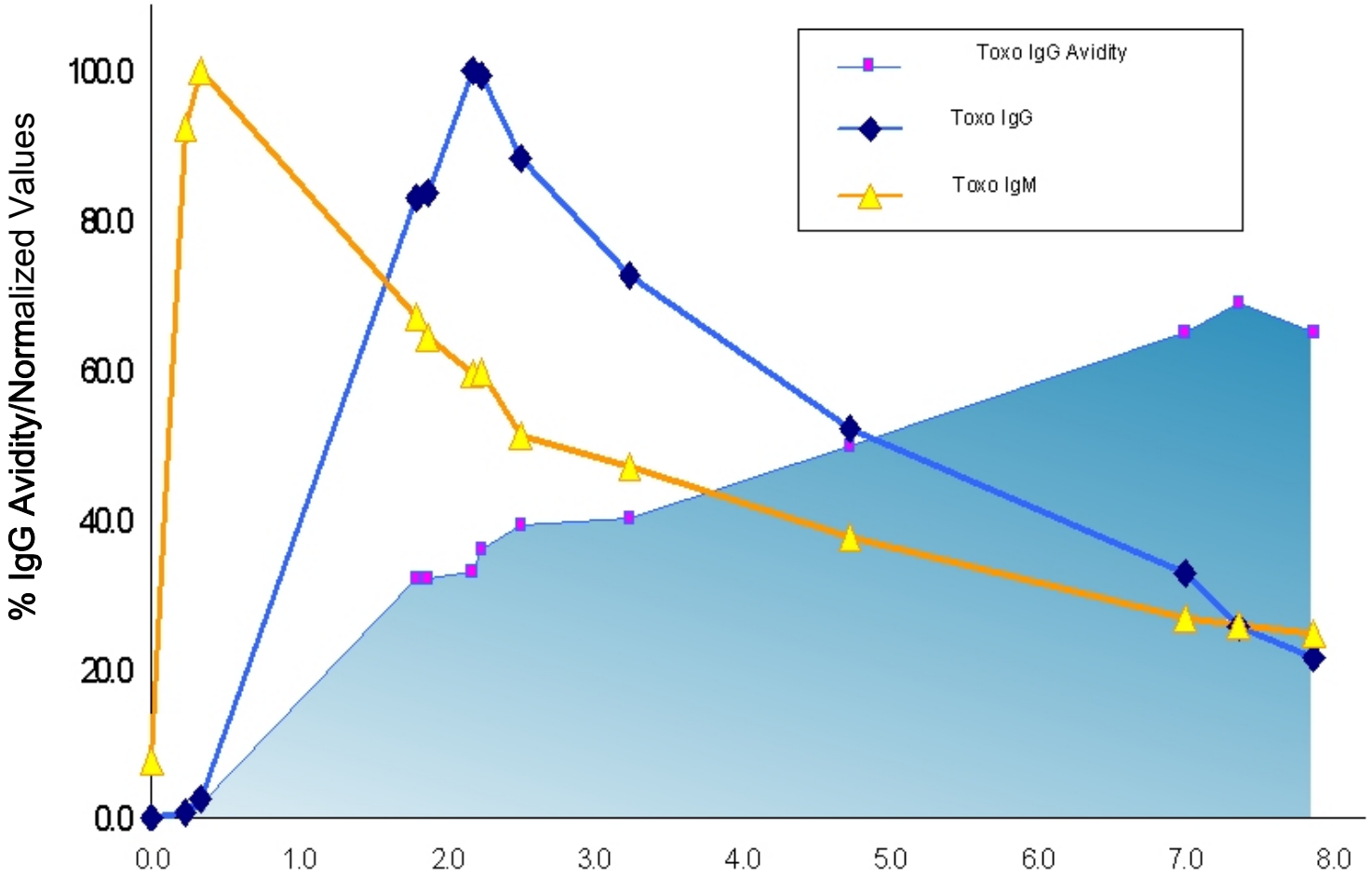
Moments

Mean	0.0434783
Std Dev	0.0260663
Std Err Mean	0.0014526
upper 95% Mean	0.0463361
lower 95% Mean	0.0406204
N	322

ARCHITECT Toxo IgM Performance Data
Seroconversion Panels

dd/mm/yy	months after first bleed	DT	HS	Isaga IgM	Vidas Toxo IgM	AxSYM Toxo IgM	ARCH Toxo IgM	ARCH Toxo IgG
28/04/2004	0.0	<2	<1	8	0.52	0.52	0.51	0.9
27/05/2004	1.0	100	8	12	7.48	6.61	8.76	44.0
8/6/2004	1.4	800	64	12	7.74	5.76	8.50	98.9
24/12/2003	0.0	<2	<1	0	0.06	0.12	0.05	0.4
24/01/2004	1.0	4	1	8	0.72	0.33	0.49	1.7
6/2/2004	1.5	20	2	12	1.01	0.36	0.65	5.2
18/02/2004	1.9	40	4	12	1.05	0.50	0.90	14.6
13/02/2004	0.0	2	<1	10	0.34	0.61	0.44	1.2
5/3/2003	0.7	10	2	12	1.81	4.22	3.05	4.9
5/11/2003	0.0	<2	<1	0		0.35	0.08	2.2
21/01/2004	2.6	200	16	12		1.56	0.92	34.8
28/01/2004	2.8	800	64	12	1.21	1.54	0.97	64.9
15/04/2004	5.4	800	64	6	0.6	0.81	0.29	57.9

ARCHITECT Toxo Assay Profile: Seroconversion Panel



Months after first ARCHITECT Toxo IgM positive bleed

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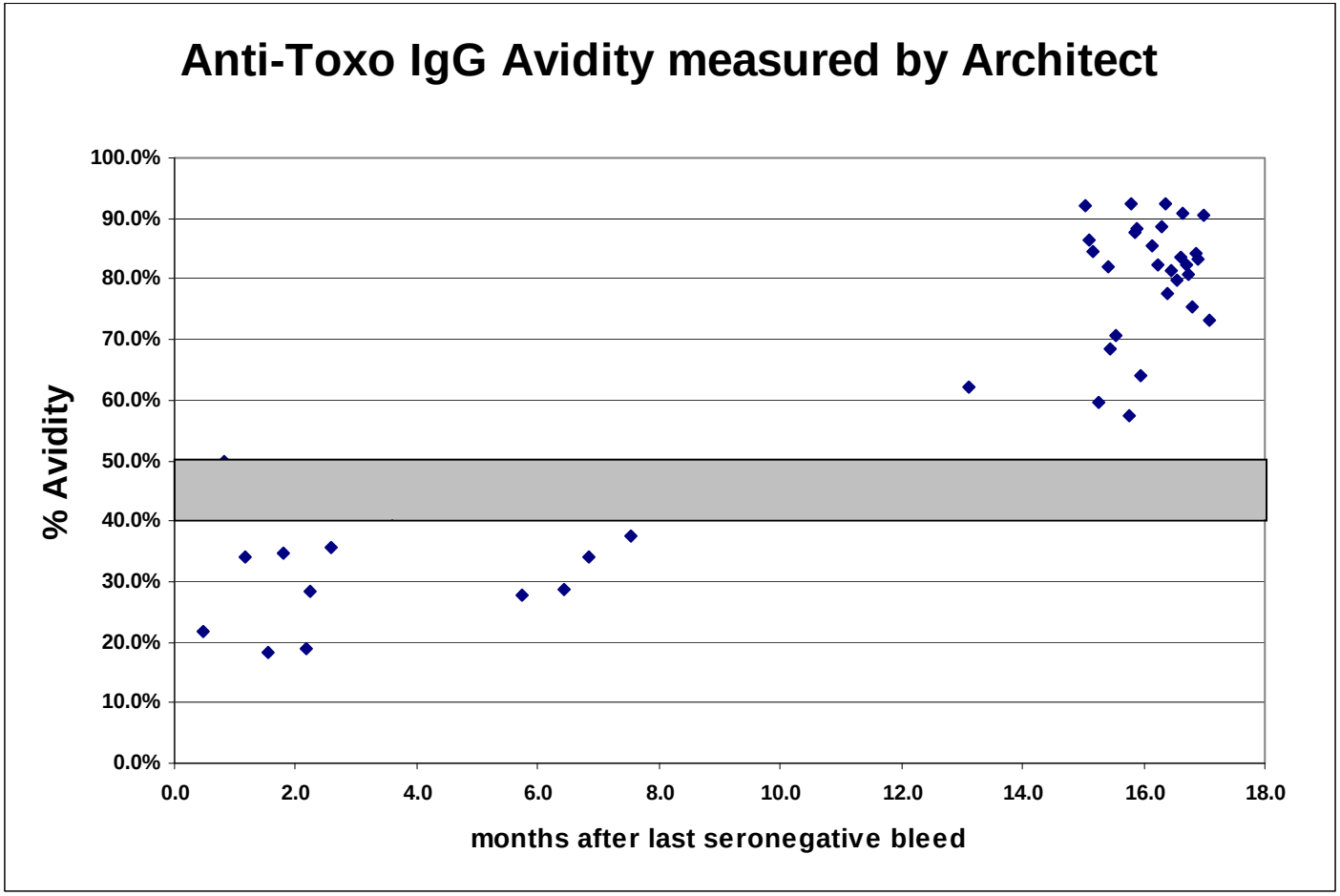
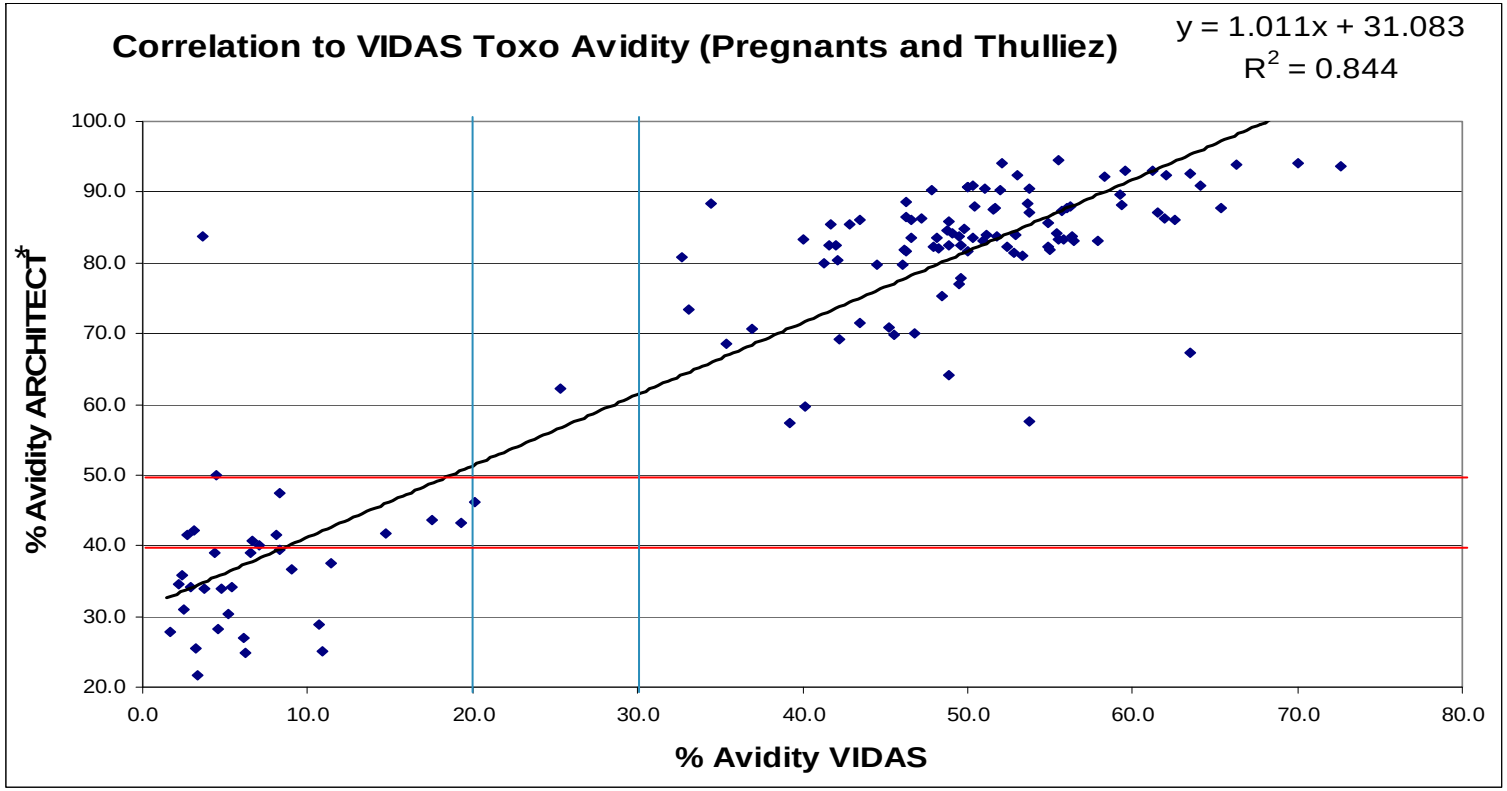
A Promise for Life

ARCHITECT Toxo IgG Avidity Profile



Architect Toxo IgG Avidity
Correlation to Vidas Toxo Avidity Assay

Samples from pregnant women
(n = 70) and seroconversion
panels (n = 63) were tested



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Summary

- Primary CMV infection and acute toxoplasmosis during gestation continue to cause fetal damage
- In the absence of a vaccine, preventative measures must be addressed
- Serologic screening can be used in a preventative manner prior to conception
- Serologic screening during gestation can be used to mitigate or prevent further damage (Toxo) or to signal the need for neonatal follow-up (CMV)