

A Chemiluminescent Microparticle CA 15-3[®] Assay* for the ARCHITECT[®] Instrument System

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Abstract (revised)

Measurement of CA 15-3 assay values has useful clinical applications for monitoring breast cancer. This abstract describes the development of an automated assay for the quantitative determination of CA 15-3 in serum for the ARCHITECT instrument system and is based on paramagnetic microparticle chemiluminescent technology. The use of the CHEMIFLEX[®] technology allows for excellent sensitivity, precision and accuracy. It is a two-step assay utilizing paramagnetic microparticles coated with the anti-CA 15-3 monoclonal antibody 115D8, an acridinium labeled anti-CA15-3 (DF3) conjugate and assay diluent. Sample, microparticles and diluent are combined in the first step. The reaction mixture is incubated. Following a wash, the DF3 conjugate is added to the mixture in the second step. Pretrigger and Trigger solutions are added to the reaction mixture. The chemiluminescence produced is measured as relative light units (RLUs). The RLUs generated are directly proportional to the

amount of CA 15-3 antigen in the sample. Preliminary performance studies with 80 replicates on 40 runs show total CVs for five panels and two controls ranging from 2.2 to 5.1. The analytical sensitivity was <0.1 U/mL. Patient samples containing high levels of CA 15-3 were serially diluted with specimen diluent. The mean dilution recovery for 3 samples is 92% with a range of 86 – 97%. Less than a 5%CV was observed for lot-to-lot reproducibility. Four hundred and two samples were analyzed on the ARCHITECT and AxSYM for the presence of CA 15-3. The regression analysis showed [ARCHITECT] = 0.941 [AxSYM] – 0.3440 (Passing-Bablok) and [ARCHITECT] = 0.922[AxSYM] + 1.7068 (least squares) with r = 0.9802.

In conclusion, an accurate, sensitive and precise CA 15-3 assay is being developed for the ARCHITECT instrument system.

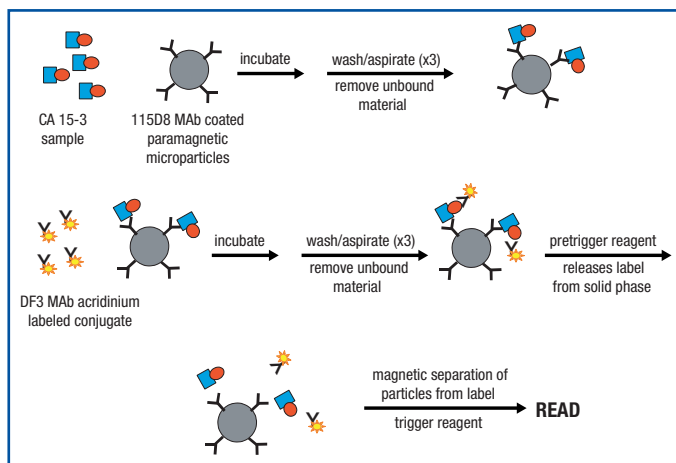
CA 15-3 Clinical Utility

Breast cancer is the second most common malignancy overall and the most common malignancy among women in the United States.¹ It is estimated that approximately 215,990 new cases of breast cancer will be diagnosed in 2004 and that approximately 40,110 women will die of the disease.¹ One in every nine women in the United States will develop breast cancer over her lifetime and approximately 30% of those women who have this malignancy will die of the disease. Metastatic disease may be present at the time of initial diagnosis and can also occur at any time following primary therapy. Up to 70% of patients with metastases will respond to systemic treatment with cytotoxic drugs or endocrine therapy; therefore, early detection of recurrence is important for effective patient management.² The median survival following diagnosis of recurrent disease is approximately 2 years, but may range from a few months to decades.^{3,4}

In patients previously treated for Stage II or Stage III breast cancer, early detection of recurrence cannot be readily accomplished by routine clinical or diagnostic studies alone. The use of a circulating serum tumor marker assay, such as the Abbott ARCHITECT CA 15-3, is useful in the identification and subsequent management of these patients.

1. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, and Thun MJ. 2004. Cancer Statistics, 2004. *CA Cancer J Clin* 54:8-29.
2. Chittoor SR and Swain SM. 1991. Adjuvant therapy in early breast cancer. *Am Fam Physician* Aug. 44(2):453-462.
3. Paterson AH, Lees AW, Hanson J, Szafran O, and Cornish F. 1980. Impact of chemotherapy of survival in metastatic breast cancer. *Lancet* Aug 9;2(8189):312.
4. Fey MF, Brunner KW, Sonntag RW. 1981. Prognostic factors in metastatic breast cancer. *Cancer Clin Trials* 4(3):237-247.

ARCHITECT CA 15-3 Assay Format



Methods

ARCHITECT CA 15-3 Assay Performance Studies

Analytical Sensitivity: The upper limit of the 95% confidence interval representing the lowest measurable concentration that can be distinguished from zero was calculated from 20 determinations.

Precision: Panels were tested in replicates of 2 on 40 runs over 20 days. The within run and total precision are expressed as %CV.

Dilution Linearity: Serum samples with elevated CA 15-3 concentrations were manually diluted using the ARCHITECT Multi-Assay Manual Diluent and then compared to the undiluted assay value.

Accuracy: Serum samples were used in a correlation to the Abbott AxSYM CA 15-3 assay. Accuracy was assessed by regression analysis.

Tumor Marker Control Comparison: BioRad Lyphocheck Controls were tested in duplicate on 10 runs over 5 days on 7 assay systems. The total %CV across all replicates on each assay is compared.

Lot-to-Lot Comparison: Panels were assayed with three different reagent lots. The three-lot comparison is expressed as the panel value %CV across the reagent lots.

Analytical Sensitivity

The analytical sensitivity, as defined by the upper limit of the 95% confidence interval representing the lowest measurable concentration that can be distinguished from zero, was calculated from 24 determinations using 10 replicates of A calibrator and 2 replicates of B calibrator per determination.

Run	Sensitivity	Run	Sensitivity	Run	Sensitivity
1	0.03	9	0.03	17	0.04
2	0.06	10	0.02	18	0.04
3	0.05	11	0.04	19	0.05
4	0.02	12	0.02	20	0.04
5	0.02	13	0.08	21	0.03
6	0.01	14	0.03	22	0.04
7	0.07	15	0.05	23	0.03
8	0.06	16	0.03	24	0.03

mean: 0.04
SD: 0.017
mean + 2SD: 0.07 U/mL

Precision

Panels were tested in replicates of 2 on 2 runs over 20 days with 2 lots.

Instrument 1: Lot 1 Instrument 2: Lot 2

	N	Mean			Within-Run			Total			
		U/mL	SD	%CV	SD	%CV	SD	%CV	SD	%CV	
panel 1	80	28.6	0.7	2.4	0.9	3.1	27.0	0.6	2.2	0.7	2.6
panel 2	80	121.2	3.6	3.0	4.5	3.7	112.6	1.9	1.7	2.4	2.2
panel 3	80	251.6	7.9	3.1	9.5	3.8	229.9	4.2	1.8	6.5	2.8
panel 4	80	494.7	18.2	3.7	22.3	4.5	477.8	12.7	2.7	17.3	3.6
panel 5	80	683.1	32.2	4.7	34.6	5.1	666.2	20.1	3.0	29.5	4.4

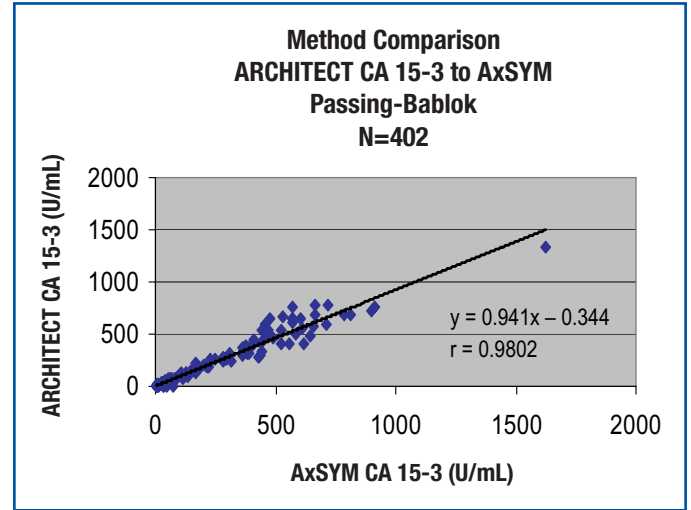
Dilution Linearity

Dilution linearity was assessed by serial manual dilution using ARCHITECT Multi-Assay Manual Diluent of three serum samples with elevated CA 15-3 concentrations.

	Dilution	Value Obtained	% Recovery	
Sample 1	undiluted	680.4		
	1:1.4	490.9	101%	
	1:2	322.7	95%	
	1:3.3	184.9	90%	
	1:5	126.8	93%	
	1:10	60.3	89%	mean
				93%
Sample 2	undiluted	754.8		
	1:1.4	505.8	94%	
	1:2	324.0	86%	
	1:3.3	198.5	87%	
	1:5	129.3	86%	
	1:10	60.7	80%	mean
				86%
Sample 3	undiluted	705.0		
	1:1.4	501.8	100%	
	1:2	352.9	100%	
	1:3.3	199.3	93%	
	1:5	140.4	100%	
	1:10	66.8	95%	mean
				97%

Accuracy

The results of the correlation analysis of samples between ARCHITECT CA 15-3 and AxSYM CA 15-3 are shown below.



Lot-to-Lot Comparison

Defibrinated plasma panels were assayed in quadruplicate across three different reagent lots.

CA 15-3 U/mL

	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5
Reagent Lot 1	25.9	106.4	215.7	436.1	602.8
Reagent Lot 2	25.9	110.7	226.3	435.8	587.8
Reagent Lot 3	25.7	107.8	218.8	439.3	592.1
Mean	25.8	108.3	220.3	437.1	594.2
%CV	0.4%	2.0%	2.5%	0.4%	1.3%

Tumor Marker Control Comparison

BioRad Tumor Controls were tested in duplicate on 10 runs over 5 days on 7 assay systems. The total %CV across all replicates on each assay is compared.

Assay System	BioRad Lyphocek 1			BioRad Lyphocek 2		
	Mean	SD	CV%	Mean	SD	CV%
Abbott ARCHITECT®	9.8	0.3	3.0	30.3	0.7	2.2
Abbott AxSYM®	12.2	0.4	2.9	35.2	1.0	2.9
Bayer ADVIA Centaur™	14.9	0.5	3.2	41.8	1.3	3.1
Beckman ACCESS™	8.4	0.2	2.9	19.1	0.8	3.9
DPC IMMULITE® 2000	16.7	0.7	4.3	44.1	1.7	3.8
OCD Vitros™ Eci	14.0	0.4	2.6	37.7	0.9	2.4
Roche Elecsys™ 2010	13.8	0.3	2.1	37.4	1.1	3.0

Conclusions

The CA 15-3 assay being developed for the ARCHITECT instrument system is:

Sensitive: analytical sensitivity less than 0.1 U/mL

Precise: total CVs less than 5.2%

Accurate: [ARCHITECT] = 0.941 [AxSYM] – 0.3440
Passing-Bablok Analysis

[ARCHITECT] = 0.922 [AxSYM] + 1.7068
Linear Regression Analysis

r value = 0.9802