

ARCHITECT® CA 125 II™: A Chemiluminescent Microparticle Assay*

J. Hofler,¹ T. Kettlety,¹ D. Wolaniuk,¹
S. Smith,¹ E. Schmidt,¹ G. Smith,¹ and T. Rosiere²

¹Fujirebio Diagnostics Inc, Malvern, PA;
²Abbott Diagnostics Division, Abbott Park, IL

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Abstract (revised)

Aims: Monitoring of ovarian cancer patients for response to therapy and for recurrence of disease is aided by CA 125 measurements. An automated method for quantitative determination of CA 125 assay values on the ARCHITECT instrument system has been developed for use with serum and plasma samples.

Methods: MAb OC 125-coated paramagnetic microparticles are combined with sample, and the OC 125-defined antigen in the sample binds to the microparticles. After washing, MAb M11 acridinium-labeled conjugate is then added. The acridinium is triggered to generate a chemiluminescent reaction. The amount of OC 125-defined antigen in the sample is proportional to the resulting chemiluminescence. The dynamic range of the assay is 0 – 1,000 U/mL.

Results: Interferences were less than 10%. Analytical sensitivity was less than 0.7 U/mL. Assay precision using defibrinated plasma-based panels between 43 and 678 U/mL yielded total CV's of 1.7 to 4.3%. Lot-to-lot reproducibility of less than 5% CV was observed. When correlated to AxSYM® CA 125, regression analysis provides the following for 279 samples: [ARCHITECT] = 1.06[AxSYM] + 4.0 (Passing-Bablok) and [ARCHITECT] = 1.00[AxSYM] + 2.3 (least squares) with $r = 0.985$.

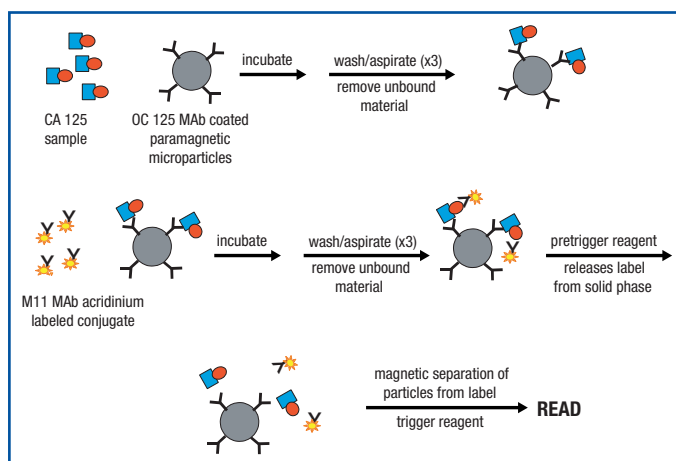
Conclusions: The ARCHITECT CA 125 II assay has been developed to provide an accurate, sensitive, and precise method for monitoring ovarian cancer patients.

CA 125 Clinical Utility

Ovarian cancer is the 12th most common malignancy overall and the 5th most common malignancy among women in the United States.¹ It is estimated that approximately 25,580 new cases of ovarian cancer will be diagnosed in 2004 and that approximately 16,090 women will die of the disease,¹ making it the leading cause of death from a gynecological cancer. The lifetime risk of development of ovarian cancer is approximately 1.5%, and one woman in 100 will die of the disease.² Serum CA 125 is the gold standard tumor marker for evaluation of pelvic masses and particularly epithelial ovarian cancer in post-menopausal women. Metastatic disease is frequently present at the time of initial diagnosis and can also occur at any time following primary therapy. A tumor marker measurement (such as the Abbott ARCHITECT CA 125 II) is therefore useful as 80% of women with advanced ovarian cancer have elevated levels of CA 125. In addition, it is valuable for secondary support for the presence of ovarian cancer pre-operatively, and it may be used postoperatively to confirm the effectiveness of therapy.³

1. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feur EJ and Thun MJ. 2004. Cancer Statistics, 2004. *CA Cancer J Clin* 54:8-29.
2. Yancik, R. 1993. Age contrasts in incidence, histology, disease stage at diagnosis, and mortality. *Cancer* 71:513-23.
3. Fishman DA and Schwartz PE. 1994. Current approaches to diagnosis and treatment of ovarian germ cell malignancies. *Curr Opinion Obstet Gynecol* 6:98-104.

ARCHITECT CA 125 Assay Format



Methods

ARCHITECT CA 125 II 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 duplicate on 40 runs over 20 non-consecutive days. The precision is expressed as %CV.

Dilution Linearity: Serum samples with elevated CA 125 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 125 assay. Accuracy was assessed by regression analysis.

Tumor Marker Control Comparison: Buffer-based panels 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	0.27	9	0.27	17	0.12
2	0.13	10	0.17	18	0.18
3	0.43	11	0.47	19	0.33
4	0.32	12	0.23	20	0.20
5	0.70	13	0.51	21	0.27
6	0.52	14	0.28	22	0.59
7	0.29	15	0.58	23	0.31
8	0.21	16	0.34	24	0.22

mean: 0.33
 SD: 0.157
 mean + 2SD: 0.64 U/mL

Precision

Panels were tested in duplicate on 40 runs over 20 days.

Instrument 1: Lot 1 Instrument 2: Lot 2

	N	Mean			Within-Run			Total			
		U/mL	SD	%CV	SD	%CV	U/mL	SD	%CV	SD	%CV
panel 1	80	39.3	1.47	3.7	1.68	4.3	39.6	0.72	1.8	1.00	2.5
panel 2	80	303.2	6.30	2.1	8.72	2.9	301.4	4.34	1.4	5.15	1.7
panel 3	80	644.3	14.58	2.3	19.14	3.0	644.1	10.82	1.7	11.67	1.8
panel 4	80	43.5	1.05	2.4	1.71	3.9	49.7	0.76	1.5	0.83	1.7
panel 5	80	303.3	9.79	3.2	11.93	3.9	340.7	5.64	1.7	6.74	2.0
panel 6	80	598.0	18.79	3.1	25.77	4.3	678.3	12.44	1.8	13.50	2.0

Panels 1 – 3 are buffer-based; panels 4 – 6 are serum-based.

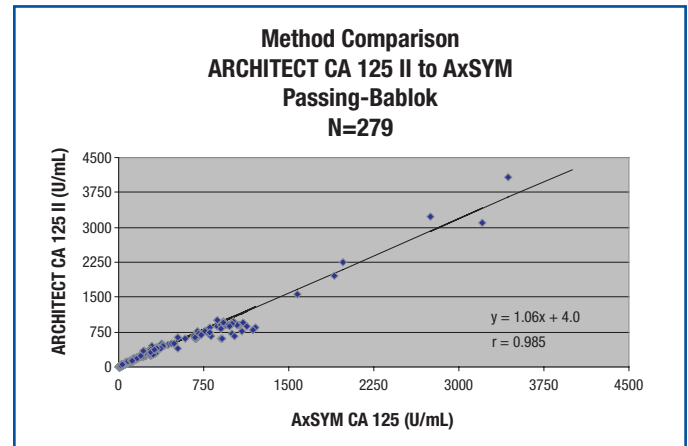
Dilution Linearity

Dilution linearity was assessed by serial manual dilution using ARCHITECT Multi-Assay Manual Diluent of four serum samples with elevated CA 125 concentrations.

	Dilution	Avg Conc.	Corrected	Recovery	
Sample 1	undiluted	846.4			
	1:1.4	631.4	884.0	104.4%	
	1:2	468.2	936.4	110.6%	
	1:3.3	282.8	933.2	110.3%	
	1:5	182.8	914.0	108.0%	
	1:10	92.7	927.0	109.5%	mean
	1:20	46.0	920.0	108.7%	108.6%
Sample 2	undiluted	903.8			
	1:1.4	631.6	884.2	97.8%	
	1:2	446.4	892.8	98.8%	
	1:3.3	274.0	904.2	100.0%	
	1:5	186.7	933.5	103.3%	
	1:10	95.4	954.0	105.6%	mean
	1:20	47.5	950.0	105.1%	101.8%
Sample 3	undiluted	935.3			
	1:1.4	645.9	904.3	96.7%	
	1:2	450.4	900.8	96.3%	
	1:3.3	284.7	939.5	100.5%	
	1:5	185.6	928.0	99.2%	
	1:10	95.8	958.0	102.4%	mean
	1:20	50.0	1000.0	106.9%	100.3%

Accuracy

The results of the correlation analysis of samples between ARCHITECT CA 125 II and AxSYM CA 125 are shown below.



Tumor Marker Control Comparison

BioRad Lyphocheck Tumor Marker Controls were assayed in duplicate on 10 runs over 5 days on 7 assay systems. The mean U/mL and %CV across all replicates on each assay is compared.

Assay System	BioRad Lyphocheck 1			BioRad Lyphocheck 2		
	Mean	SD	CV%	Mean	SD	CV%
Abbott ARCHITECT®	43.4	1.4	3.2	134.6	3.5	2.6
Abbott AxSYM®	42.1	3.3	7.8	135.2	8.3	6.2
Bayer ADVIA Centaur™	39.7	1.2	3.1	129.6	4.4	3.4
Beckman ACCESS™	29.5	0.6	1.9	103.1	3.0	2.9
DPC IMMULITE® 2000	28.2	1.1	3.8	99.5	3.2	3.2
OCD Vitros™ Eci	27.8	0.5	1.8	95.7	2.0	2.1
Roche Elecsys™ 2010	34.0	0.6	1.8	100.5	1.8	1.8

Lot-to-Lot Comparison

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

CA 125 II U/mL

	Panel 1	Panel 2	Panel 3
Reagent Lot 1	45.0	303.9	601.8
Reagent Lot 2	43.3	308.5	601.4
Reagent Lot 3	43.5	301.3	594.0
Mean	43.9	304.6	599.1
%CV	2.1%	1.2%	0.7%

Conclusion

The ARCHITECT CA 125 II assay is:

Sensitive: analytical sensitivity less than 0.70 U/mL

Precise: total CVs less than 4.5%

Accurate: $[\text{ARCHITECT}] = 1.06[\text{AxSYM}] + 4.0$
(Passing-Bablok)

$[\text{ARCHITECT}] = 1.00[\text{AxSYM}] + 2.3$
(Least Squares)

$r = 0.985$