

A Chemiluminescent Microparticle Squamous Cell Carcinoma Associated Antigen Assay* for the ARCHITECT[®] Instrument System

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Abstract (revised)

Measurement of squamous cell carcinoma associated antigen (SCC Ag) is widely used for diagnosis and monitoring of cervical, lung and other types of squamous cell carcinoma. An assay for SCC is being developed to run on the ARCHITECT instrument system to provide more precise and convenient testing of the SCC Ag.

ARCHITECT SCC is an automated assay for the quantitative determination of SCC Ag in serum or plasma and is based on paramagnetic microparticle chemiluminescent (CHEMIFLEX[®]) technology. It is a two-step assay utilizing microparticles coated with the anti-SCC Ag monoclonal antibody (MAb) and an acridinium labeled anti-SCC Ag MAb. Sample and microparticles are combined in the first step, incubated and washed, and then the conjugate is added in the second step. After washing, Pretrigger

and Trigger are added to produce chemiluminescence, which is measured as relative light units (RLUs). The RLUs correspond to the concentrations of the SCC Ag in the sample. The ARCHITECT SCC assay has a dynamic range of 0.1 – 70 ng/mL with ability to run up to 200 tests/hour on the ARCHITECT instrument.

ARCHITECT SCC showed the following performance characteristics: Analytical sensitivity was ≤ 0.1 ng/mL. Total variability (%CV by NCCLS protocol EP5-A) was $\leq 6\%$. Good dilution linearity, good spike recovery, no significant carryover, and good correlation ($r > 0.99$) with IMx[®] SCC (Abbott) were demonstrated. For apparently healthy subjects, 95.1% were below the cutoff of 1.5 ng/mL. The ARCHITECT SCC assay is a sensitive, precise, accurate and convenient SCC Ag test being developed for the ARCHITECT instrument system.

SCC Clinical Utility^{1,2}

Squamous cell carcinoma associated antigen (SCC Ag) is a subfraction of TA-4, a tumor associated antigen first described by Kato and Torigoe in 1977. TA-4, obtained from squamous cell cancer tissue of the uterine cervix, has been characterized as a glycoprotein with a molecular weight of 48,000 daltons.

Two genes for SCC Ag (SCC Ag-1 and SCC Ag-2) have been identified. Their products are highly homologous and classified as serine protease inhibitors (serpin). SCC Ag-1 inhibits apoptosis of tumor cells induced by anticancer drug. Therefore SCC Ag-1 may work in cancer cells for tumor growth, and in normal squamous epithelium for differentiation by means of the inhibition of apoptosis. Recombinant SCC Ag-2 inhibits cathepsin G and mast cell chymase, suggesting that it protects epithelial cells from the inflammation induced by these proteases.

Early studies showed that TA-4 serum levels in women with cervical squamous cell cancer were frequently elevated above those found in healthy individuals. Other studies have indicated serum TA-4 levels may reflect the extent of disease in women with cervical squamous cell cancer and that TA-4 levels could be useful as an adjunct in predicting prognosis, detecting recurrence and monitoring disease status.

SCC antigen has been studied in other squamous cell malignancies including lung, esophagus, head and neck, anal canal, and skin. In general, a pattern similar to that seen with squamous cell carcinoma of the uterine cervix is seen with these tumors, (i.e., the more advanced tumor stages are reportedly associated with higher SCC antigen levels).

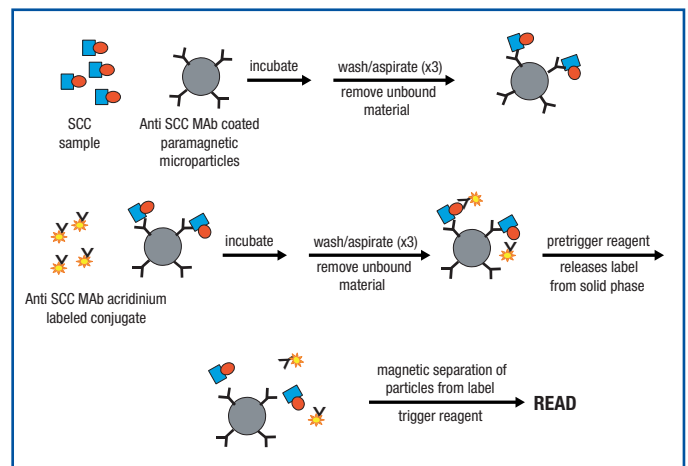
Researchers have reported that measurement of the antigen, in serial determinations, may indicate disease recurrence, residual disease following treatment, and response to therapy.

Serial SCC Ag measurements lead to earlier detection of recurrent disease after surgery for cancer of the uterine cervix. In a prospective follow-up study, 47 of the 55 patients (86%) with recurrent disease demonstrated elevated serum SCC at the time recurrence or disease progression was recognized. The profile of serum SCC Ag parallels the response to radiotherapy and provides a way of evaluation the effectiveness of chemotherapy.

For patients with cervical cancer, SCC Ag is a more sensitive marker for detecting recurrence or progression than CYFRA-21.1 and TPS (56 and 40%, respectively, compared to 75% for SCC Ag).

ARCHITECT SCC

Assay Format



Methods

Studies were conducted to characterize the performance of the ARCHITECT SCC assay (in development).

Analytical Sensitivity: The upper limit of the 95% confidence interval representing the lowest measurable concentration that can be distinguished from zero (rep=10) was calculated.

Precision: Panels and controls were tested following NCCLS protocol EP5-A. The within run and total precision are expressed as %CV.

Dilution Linearity: Serum samples with elevated SCC concentrations were manually diluted using the Calibrator A and then compared to the undiluted assay value.

Spike Recovery: SCC antigen was spiked in serum and plasma samples. Recovery (%) was calculated using the Calibrator A as the control.

Correlation: Serum samples were used in a correlation study versus the Abbott IMx SCC assay. Correlation was assessed by Passing-Bablok analysis.

Normal Range: Serum samples from apparently healthy subjects were tested with IMx SCC and ARCHITECT SCC.

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 tested with 12 determinations (3 lots of reagent, 2 instrument and 2 runs each). 10 replicates of A calibrator and 4 replicates of B calibrator were used per determination.

Instrument	Reagent Lot	Run	Analytical Sensitivity (ng/mL)
Instrument 1 (i201201)	Lot 1	Run 1	0.074
		Run 2	0.076
	Lot 2	Run 1	0.058
		Run 2	0.079
	Lot 3	Run 1	0.077
		Run 2	0.069
Instrument 2 (i201861)	Lot 1	Run 1	0.058
		Run 2	0.020
	Lot 2	Run 1	0.040
		Run 2	0.036
	Lot 3	Run 1	0.046
		Run 2	0.072

Mean: 0.059 ng/mL

Precision

Panels and controls were tested following NCCLS protocol EP5-A (20 days, 2 runs/day, 2 replicates). 3 lots of reagent were used.

Sample	Mean (ng/mL)	Reagent Lot 1		Reagent Lot 2		Reagent Lot 3	
		Total CV%	Within Run CV%	Total CV%	Within Run CV%	Total CV%	Within Run CV%
Control-L	2.0	5.05%	4.31%	3.97%	3.30%	5.60%	4.63%
Control-M	10.0	5.01%	3.83%	4.56%	4.60%	4.72%	4.91%
Control-H	50.0	5.26%	4.74%	4.36%	4.25%	4.46%	3.88%
Panel-1	1.5	5.92%	5.02%	5.43%	4.96%	5.63%	5.08%
Panel-2	7.4	4.79%	3.74%	5.21%	4.03%	4.67%	3.94%
Panel-3	58.5	4.37%	3.79%	4.40%	3.84%	3.95%	3.79%

Controls are buffer-based; Panels are serum-based.

Dilution Linearity

Dilution linearity was assessed by serial manual dilution using Calibrator A of five elevated SCC serum samples.

Sample	Dilution Factor	ng/mL		Recovery	
		Avg Conc.	Corrected		
Sample 1	1:1	41.9	41.9		
	1:2	22.6	45.2	107.7%	
	1:4	10.9	43.6	104.0%	
	1:8	5.5	43.7	104.3%	
	1:16	2.6	41.3	98.5%	Mean
	1:32	1.3	40.3	96.0%	102.1%
Sample 2	1:1	46.7	46.7		
	1:2	23.6	47.3	101.3%	
	1:4	11.6	46.3	99.3%	
	1:8	5.7	45.7	97.8%	
	1:16	2.9	45.9	98.4%	Mean
	1:32	1.3	40.2	86.1%	96.6%
Sample 3	1:1	45.7	45.7		
	1:2	21.7	43.5	95.1%	
	1:4	12.0	47.9	104.8%	
	1:8	5.3	42.7	93.4%	
	1:16	2.7	42.6	93.3%	Mean
	1:32	1.3	40.2	87.9%	94.9%
Sample 4	1:1	50.6	50.6		
	1:2	26.9	53.7	106.3%	
	1:4	13.9	55.4	109.6%	
	1:8	6.9	55.4	109.5%	
	1:16	3.3	52.8	104.5%	Mean
	1:32	1.7	54.3	107.4%	107.4%
Sample 5	1:1	45.9	45.9		
	1:2	25.0	50.0	108.9%	
	1:4	12.5	49.9	108.7%	
	1:8	6.0	48.0	104.5%	
	1:16	3.0	47.9	104.4%	Mean
	1:32	1.4	45.0	98.2%	104.9%

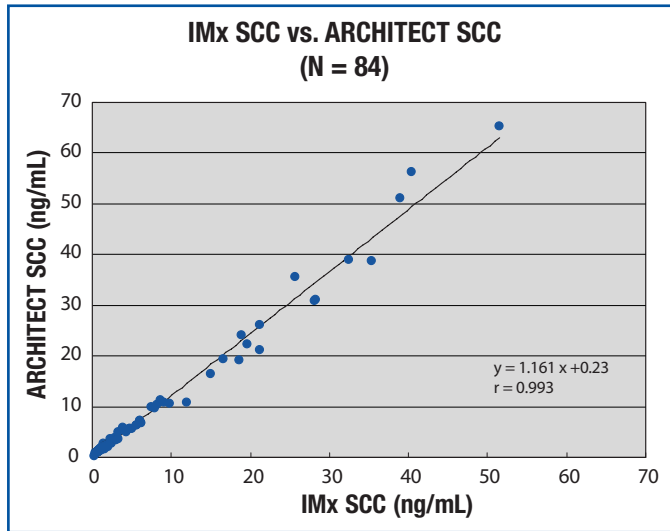
Spike Recovery

Two levels (10 ng/mL and 50 ng/mL) of SCC antigen was spiked in 5 serum and 5 plasma samples. Recovery (%) was calculated using the Calibrator A as the control.

Base Sample	Spiked Level	ng/mL Mean	Recovered Value	% Recovery	
Cal A	10	10.09		100.0%	
	50	52.53		100.0%	
Normal Serum 1	0	0.38			
	10	10.35	9.98	98.9%	
	50	49.53	49.16	93.6%	
Normal Serum 2	0	0.35			
	10	10.67	10.32	102.2%	
	50	52.48	52.13	99.2%	
Normal Serum 3	0	0.37			
	10	10.90	10.53	104.3%	
	50	51.10	50.74	96.6%	
Normal Serum 4	0	0.25			
	10	10.14	9.89	98.0%	
	50	48.29	48.04	91.5%	
Normal Serum 5	0	0.31			
	10	10.48	10.16	100.7%	mean
	50	51.65	51.33	97.7%	98.3%
Cal A	10	10.05		100.0%	
	50	50.82		100.0%	
Normal Plasma 1	0	0.49			
	10	10.50	10.01	99.6%	
	50	50.66	50.17	98.7%	
Normal Plasma 2	0	0.44			
	10	9.88	9.44	93.9%	
	50	46.85	46.41	91.3%	
Normal Plasma 3	0	0.41			
	10	10.11	9.70	96.5%	
	50	49.17	48.75	95.9%	
Normal Plasma 4	0	0.49			
	10	10.45	9.96	99.1%	
	50	50.64	50.16	98.7%	
Normal Plasma 5	0	0.48			
	10	10.00	9.52	94.7%	mean
	50	49.65	49.17	96.7%	96.5%

Correlation

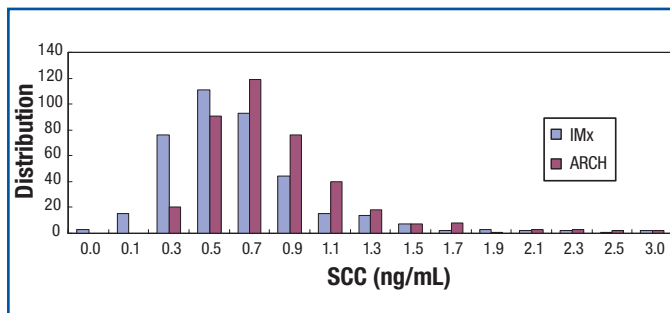
84 serum samples were used in a correlation study to the Abbott IMx SCC assay. Correlation was assessed by Passing-Bablok analysis.



Normal Range

390 serum samples from apparently healthy subjects were tested. 95.13% of the samples ≤ 1.5 ng/mL with ARCHITECT SCC. When 1.5 ng/mL is used as "cutoff", agreement with IMx SCC was 97.7%.

IMx SCC			ARCHITECT SCC			ARCHITECT			
ng/mL	Samples	Cum. %	ng/mL	Samples	Cum. %	> 1.5	≤ 1.5	SUM	
≤ 0.50	205	52.56%	≤ 0.50	111	28.46%	> 1.5	≤ 1.5	SUM	
0.5 – ≤ 1.0	144	89.49%	0.5 – ≤ 1.0	213	83.08%	IMx > 1.5	11	1	12
1.0 – ≤ 1.5	29	96.92%	1.0 – ≤ 1.5	47	95.13%	IMx ≤ 1.5	8	370	378
1.5 – ≤ 5.0	12	100.00%	1.5 – ≤ 5.0	19	100.00%	IMx SUM	19	371	390
> 1.5 ng/mL	3.08%		> 1.5 ng/mL	4.87%		Agreement 97.69%			



Conclusion

The SCC assay being developed for the ARCHITECT instrument system is:

Sensitive: analytical sensitivity less than 0.1 ng/mL

Precise: total CVs less than 6%

Accurate: good dilution linearity and spike recovery
good correlation with IMx ($r > 0.99$)

Convenient: up to 200 tests/hour on ARCHITECT

References

¹Sumitani Y, Nawata S, and Kato H. Biological Role of SCC Antigen. *Tumor Biology* 1998; 19:488-493.

²de Bruijn HWA, Duk JM, van der Zee AGJ, Pras E, Willemse PHB, Boonstra H, Hollema H, Mourits MJE, de Vries EGE, and Aalders JG. The Clinical Value of Squamous Cell Carcinoma Antigen in Cancer of the Uterine Cervix. *Tumor Biology* 1998;19:505-516.