

An Enhanced Chemiluminescent Microparticle CA 19-9TM Assay* for the ARCHITECT[®] Instrument System

G.A. Smith¹, W.E. Covert¹, and S.B. O'Morchoe²

¹*Fujirebio Diagnostics Inc, Malvern, PA;*

²*Abbott Diagnostics Division, Abbott Park, IL*

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Abstract (revised)

Aims

To develop an enhanced ARCHITECT CA 19-9_{XR} assay.

Methods

The ARCHITECT CA 19-9 assay, List No. 6C04, has a dynamic range of 0 to 500 U/mL. The enhanced ARCHITECT CA 19-9_{XR} assay, List No. 2K91, will incorporate an extended dynamic range of 0 to 1000 U/mL. The assay is a two-step assay utilizing paramagnetic microparticles coated with monoclonal antibody 1116-NS-19-9, an acridinium labeled monoclonal antibody 1116-NS-19-9 conjugate and assay diluent. The chemiluminescence produced is measured as relative light units (RLUs). The RLUs generated are directly proportional to the amount of 1116-NS-19-9 reactive determinants in the sample. Standard calibrators at 0, 30, 100, 250, 500 and 1000 U/mL were prepared by correlation with the Fujirebio CA 19-9 RIA assay.

Results

Preliminary performance studies using 2 reagent lots to assay 2 replicates at two separate times per day for ten days (n = 40 for each sample for each lot) show total CVs that range from 5.2% to 8.2% and within run CVs that range from 4.7% to 8.1%. The analytical sensitivity was <2.0 U/mL. Four hundred fifty-one samples were analyzed on the ARCHITECT and RIA CA 19-9 assays for the presence of 1116-NS-19-9 reactive determinants. The calculated linear regression produced a slope of 1.04, an intercept of -1.93 and an r-value of 0.95.

Conclusions

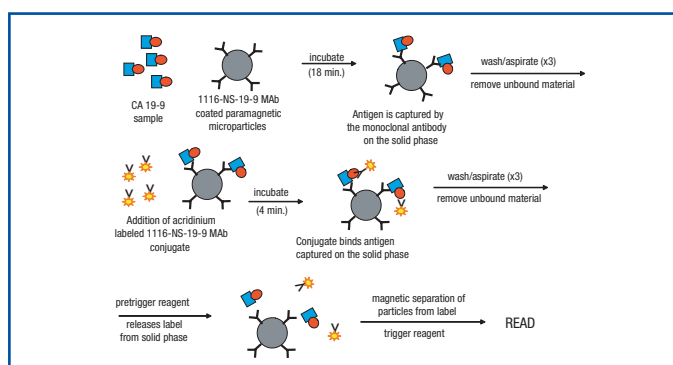
The enhanced ARCHITECT CA 19-9_{XR} assay is accurate, sensitive and precise with the advantage of an extended dynamic range.

CA 19-9 Clinical Utility

In general, cancer tumor markers are useful as adjunctive tests to manage cancer patients in whom changing levels are observed. Carbohydrate antigen 19-9 (CA 19-9), the sialylated Lewis^a blood group antigen defined by the monoclonal antibody 1116 NS 19-9¹ is present in the epithelium of the fetal stomach, intestine, liver and pancreas and in the sera of cancer patients.^{2,3} It is primarily used as a marker for pancreatic cancer. Elevated serum levels, >37 U/mL⁴, are also seen with various gastrointestinal malignancies, such as colorectal, gastric and hepatic carcinomas. CA 19-9 is useful in post-therapeutic monitoring to determine the success of therapy or the development of recurrence when used serially.

1. Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D and Fulner P. 1979. Colorectal carcinoma antigens detected by hybridoma antibodies. *Somatic Cell Genet* 5:957-971.
2. Halm U, Schumann T, Schiefke I, Witzigmann H, Mossner J and Keim V. 2000. Decrease of CA 19-9 during chemotherapy with gemcitabine predicts survival time in patients with advanced pancreatic cancer. *British Journal of Cancer* 82:1013-1016.
3. Nakao A, Oshima K, Nomoto S, Takeda S, Kaneki T, Ichihara T, Kurokawa T, Nonami T and Takagi H. 1998. Clinical usefulness of CA 19-9 in pancreatic carcinoma. *Semin Surg Oncol* 15:15-22.
4. Montgomery R, Hoffman J, Riley L, Rogatki A, Ridge J and Eisenberg B. 1997. Prediction of recurrence and survival by post-resection CA 19-9 values in patients with adenocarcinoma of the pancreas. *Ann Surg Oncol* 4:551-556.

ARCHITECT CA 19-9_{XR} Assay Format



Methods

Studies were conducted to characterize the performance of the ARCHITECT CA 19-9_{XR} assay.

Analytical Sensitivity

The upper limit of the 95% confidence interval representing the lowest measurable concentration that can be distinguished from zero was calculated from 18 determinations.

Precision

Controls and panels were tested in replicates of two at two separate times per day for ten days (n = 40 for each sample). The within run and total precision are expressed as %CV.

Accuracy

Serum samples were used in a correlation to the Fujirebio CA 19-9 RIA assay. Accuracy was assessed by linear regression analysis, (Passing-Bablok).

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 18 determinations. Using 2 reagent lots, 3 assays consisting of 10 replicates of A calibrator and 2 replicates of B calibrator per assay were run on 3 different instruments.

	Instr. 1	Instr. 2	Instr. 3
Reagent Lot 1, Run 1	1.00	0.87	0.64
Reagent Lot 1, Run 2	1.08	1.18	0.27
Reagent Lot 1, Run 3	0.74	1.25	1.00
Reagent Lot 2, Run 1	0.82	1.39	0.68
Reagent Lot 2, Run 2	1.23	1.89	1.42
Reagent Lot 2, Run 3	1.09	1.06	0.82

Mean = 1.02

SD = 0.38

Mean + 2SD = 1.78 U/mL

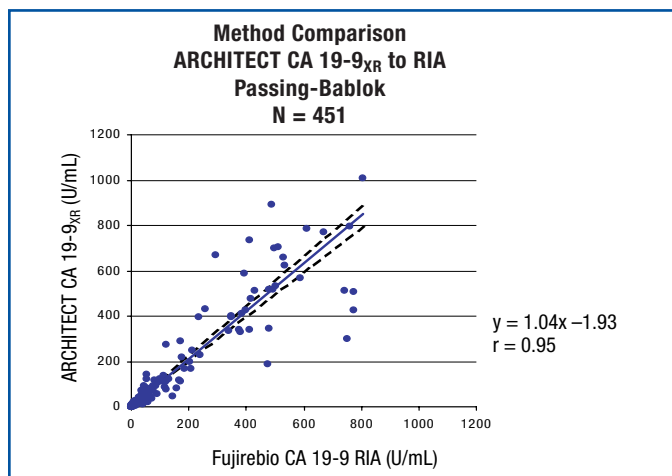
Precision

Three levels of buffer based assay controls and 3 samples that were prepared by spiking circulating 1116-NS-19-9 determinants into normal human serum were assayed on each of 10 days using 2 reagent lots in replicates of 2. Two assays were performed each day.

Lot	Sample	N	Mean CA 19-9 U/mL	Within Run %CV	Total %CV
1	Low Control	40	54.08	7.9	8.2
	Medium Control	40	134.10	4.7	5.5
	High Control	40	248.08	5.3	5.3
	Low Panel	40	41.45	8.1	8.1
	Medium Panel	40	376.51	5.1	5.3
	High Panel	40	597.42	5.1	5.2
2	Low Control	40	49.47	7.5	7.5
	Medium Control	40	126.11	5.3	5.3
	High Control	40	232.54	5.3	5.5
	Low Panel	40	40.31	6.2	6.2
	Medium Panel	40	377.77	5.8	5.9
	High Panel	40	597.31	5.1	5.3

Accuracy

The results of the correlation analysis of sample values determined with the ARCHITECT CA 19-9_{XR} assay compared to sample values determined with the Fujirebio CA 19-9 RIA assay are shown below.



Conclusions

The CA 19-9_{XR} assay being developed for the ARCHITECT instrument system is:

- Sensitive: analytical sensitivity less than 2.0 U/mL
- Precise: total CVs less than 8.3%
- Accurate: [ARCHITECT] = 1.04 [RIA] - 1.93, $r = 0.95$

