

Quantitation of Tumor Markers by LOCI™ Reagent Technology on the Dimension Vista™ Intelligent Lab System

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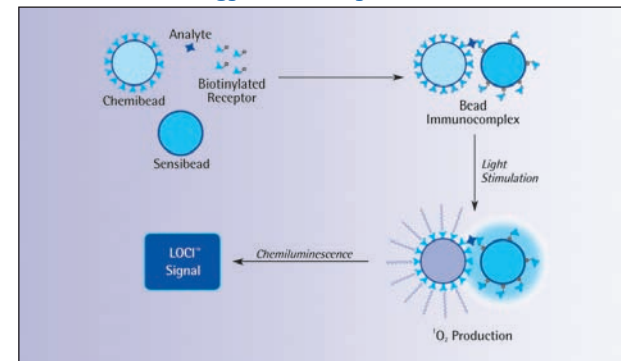
Abstract

LOCI™ advanced chemiluminescence technology enables high sensitivity immunoassay formats. LOCI technology incorporates two bead reagents, a sensibead and a chemibead, and a biotinylated monoclonal antibody fragment. The sensibead reagent contains streptavidin and photosensitizer dye. The chemibead reagent contains a monoclonal antibody and chemiluminescent dye. Immunocomplexes form in the presence of analyte. Illumination of the complex generates singlet oxygen from sensibeads, which diffuses into chemibeads triggering a chemiluminescent reaction. We describe the development and initial analytical performance of sandwich immunoassays for measurement of alpha-fetoprotein (AFP)*, breast cancer associated antigen (BCAA)*, carbohydrate antigen 125 (CA 125)* and carcinoembryonic antigen (CEA)* in serum on the Dimension Vista™ System*. Turnaround time is 10 minutes for all methods. Precision estimates were obtained per CLSI EP5A-2 using quality control materials or human serum pools. Method comparisons with patient specimens were conducted versus methods on the Bayer ADVIA Centaur® Immunoassay System for AFP, the Beckman Coulter Access® Immunoassay System for CEA and the Roche Elecsys® System for CA 15-3™ II and CA 125.

No significant interference (<10% bias) was seen from lipemia (2650 mg/dL triglycerides), cholesterol (500 mg/dL), hemoglobin (250 mg/dL), or icterus (20 mg/dL conjugated bilirubin or 20 mg/dL unconjugated bilirubin). In conclusion, the use of LOCI™ technology demonstrated excellent specificity, sensitivity, precision, turnaround time and dynamic range suitable for the measurement of tumor markers such as AFP, BCAA, CA125 and CEA on the Dimension Vista™ System.

Assay	Sample Volume (µL)	Precision (% CV)		Assay Range	Method Comparison (X-axis, Access_Centaur or Elecsys)			
		Repeatability	Within Laboratory		Slope	Intercept	r	N
AFP	2.0	2.3-2.7	2.4-2.7	0.2-1000.0 ng/mL	0.945 (C)	2.1	0.99	60
BCAA	2.0	1.1-2.0	2.8-3.0	1.0-300.0 U/mL	0.968 (E)	30.2	0.95	68
CA 125	10.0	1.0-2.9	1.4-3.3	2.0-5000.0 U/mL	0.988 (E)	-69	0.98	57
CEA	2.0	1.6-1.8	2.3-3.2	0.2-1000.0 ng/mL	0.971 (A)	4.31	0.99	49

LOCI™ Technology and Assay Format



Chemibeads Latex beads (200 nm) contain an olefin dye that reacts with singlet oxygen to form a O_2 adduct, which decays and generates the chemiluminescent signal. The beads also contain a fluorescent energy acceptor that shifts the emission wavelength to 612 nm. A blocking layer surrounds the beads to isolate the label and minimize potential nonspecific binding. In the sandwich format, capture antibodies are bound to the Chemibead surface.

Sensibeads Latex beads (200 nm) contain a photosensitive dye that absorbs light at 680 nm and generates singlet oxygen (1O_2). A blocking layer surrounds the beads to minimize potential nonspecific binding. Streptavidin, conjugated to the bead surface, binds to the biotinylated receptor reagent. This brings the singlet oxygen source (Sensibead) in close proximity of the singlet oxygen receptor (Chemibead) to form the bead pair immunocomplex for LOCI™ signal generation.

Biotinylated Receptors Analyte specific reagents, typically a biotinylated antibody. They serve as part of the bridge between Chemibead and Sensibead in the bead pair immunocomplex.

Method Specifics

	AFP Alpha-fetoprotein	BCAA Breast cancer associated antigen	CA 125 Carbohydrate antigen 125	CEA Carcinoembryonic antigen
Assay time	10 min.	10 min.	10 min.	10 min.
Assay range	0.2-1200 ng/mL	1.0-300.0 U/mL	2.0-5000 U/mL	0.2-1000 ng/mL
Sample volume	2.0 µL	2.0 µL	10.0 µL	2.0 µL
Interferences [§]				
Bilirubin, mixed isomer	60 mg/dL	35.2 mg/dL	39.1 mg/dL	60 mg/dL
Bilirubin, dtauro	60 mg/dL	60 mg/dL	20 mg/dL	60 mg/dL
Hemoglobin	1000 mg/dL	585 mg/dL	250 mg/dL	1000 mg/dL
Triglycerides	3000 mg/dL	2650 mg/dL	2650 mg/dL	3000 mg/dL
Cholesterol	500 mg/dL	500 mg/dL	500 mg/dL	500 mg/dL

[§] bias <10% for test compounds at least up to concentrations listed

Reproducibility

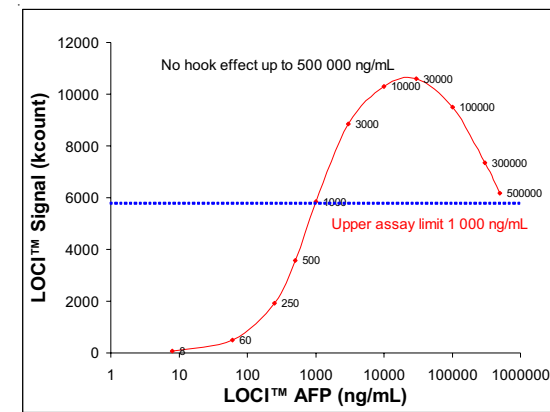
Test samples were processed in duplicate daily for 10 (AFP) or 20 days (other) using a prototype Dimension Vista™ system. The results were treated by analysis of variance (ANOVA) to yield reproducibility estimates. The QC material was Bio-Rad Maternal Serum Control (AFP), Bio-Rad Lyphochek™ Immunoassay Plus (CEA) or serum pools (other).

	Mean	Precision (% CV)	
		Repeatability	Within Laboratory
AFP (ng/mL)			
BioRad Control 1	9.9	2.3	2.4
BioRad Control 2	32.0	2.3	2.6
BioRad Control 3	90.1	2.7	2.7
BCAA (U/mL)			
Serum pool	12.8	1.1	7.3
Serum pool	39.7	1.3	2.9
Serum pool	79.8	2.0	2.8
CA 125 (U/mL)			
Serum pool	11.1	7.2	8.6
Serum pool	39.7	1.3	2.9
Serum pool	107.8	1.7	2.8
Serum pool	2608	0.53	0.76
CEA (ng/mL)			
Serum pool	3.8	1.6	3.2
Serum pool	15.0	1.8	3.0
Serum pool	76.4	1.6	2.3
BioRad Control 1	2.2	2.0	3.7
BioRad Control 2	17.4	1.5	1.9
BioRad Control 3	38.1	1.6	1.7

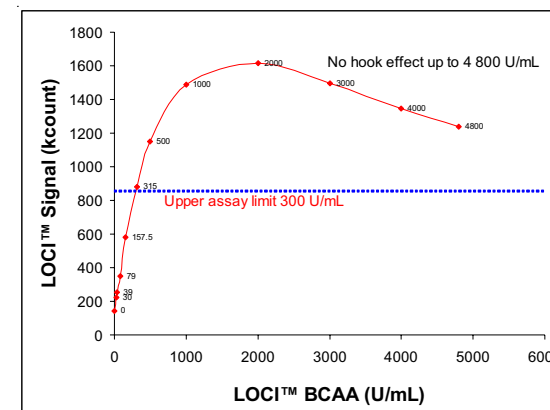
High Dose Hook Effect

AFP, mucin 1 (MUC 1; BCAA), CA 125 or CEA antigen was spiked into aliquots of either 6% bovine serum albumin in a HEPES buffer (AFP and CEA) or pooled human serum (BCAA and CA 125) and processed in triplicate along with the method calibrators by each LOCI™ method on a prototype Dimension Vista™ system. In all cases no high dose hook effect was observed.

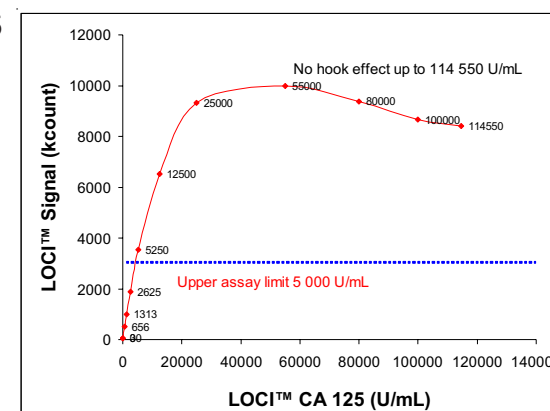
AFP



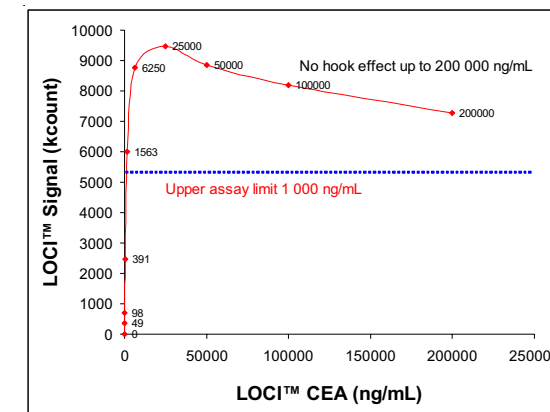
BCAA



CA 125



CEA



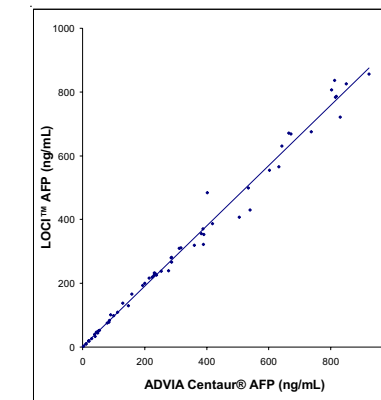
Method Comparison

The LOCI™ methods processed on a prototype Dimension Vista™ system were compared with the Bayer ADVIA Centaur® Immunoassay System for AFP, the Beckman Coulter Access® Immunoassay System for CEA and the Roche Elecsys® System for CA 15-3™ II and CA 125 through a patient sample correlation study. The results were analyzed by linear regression.

AFP

Linear Regression

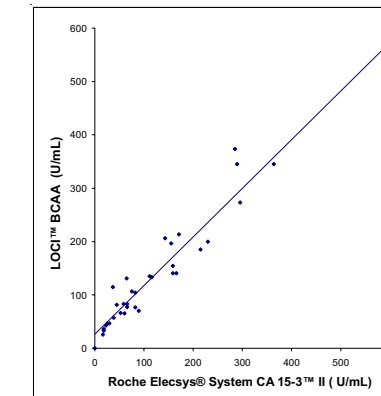
slope 0.945
Intercept 2.078
r 0.994
n 60



BCAA

Linear Regression

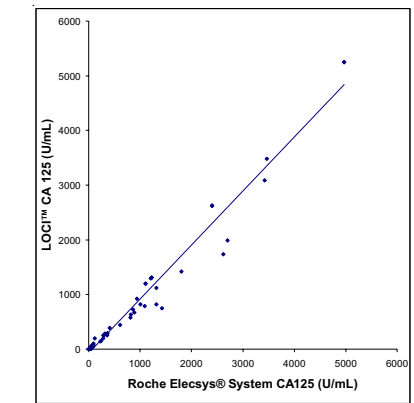
slope 0.968
Intercept 30.2
r 0.984
n 68



CA 125

Linear Regression

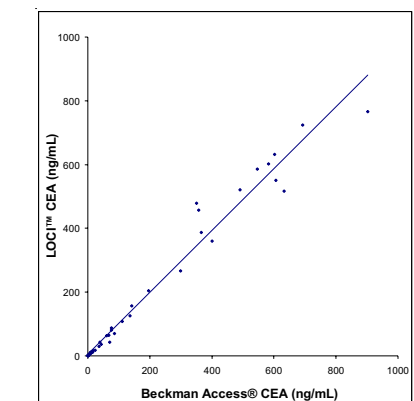
slope 0.988
Intercept -69
r 0.984
n 57



CEA

Linear Regression

slope 0.971
Intercept 4.311
r 0.986
n 49



Conclusions

- Methods for tumor markers alpha-fetoprotein (AFP)*, breast cancer associated antigen (BCAA)*, carbohydrate antigen 125 (CA 125)* and carcinoembryonic antigen (CEA)* were developed using reagents based upon LOCI™ technology and processed with a prototype Dimension Vista™ system*
- The methods demonstrated acceptable assay ranges, reproducibility, and turnaround time
- The methods showed good agreement with their counterparts on the Roche Elecsys® System, Bayer ADVIA Centaur® Immunoassay System and Beckman Coulter® Access Immunoassay System
- Each method demonstrated minimal hook effect
- Each method demonstrated no significant interference from lipemia (2650 mg/dL triglycerides), cholesterol (500 mg/dL), hemoglobin (250 mg/dL), or icterus (20 mg/dL conjugated bilirubin or 20 mg/dL unconjugated bilirubin)

*Products under development – not available for sale