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Rapid and Cost Effective Immunoassay Development and Validation of a Biomarker Assay Using Spatial Proximity Analyte Reagent Capture Luminescence, SPARCL™

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ABSTRACT

A novel detection technology was used for rapid immunoassay (ligand binding assay) development and validation. SPARCL (Spatial Proximity Analyte Reagent Capture Luminescence) technology is a proximity dependent, homogeneous, chemiluminescent detection method. In a SPARCL assay, a chemiluminescent substrate (acridan) is brought into the proximity of an oxidative enzyme (horseradish peroxidase) through the specific antigen/antibody interaction. A flash of light proportional to the quantity of analyte present in the sample is generated upon addition of a trigger solution containing H_2O_2. Commercially sourced reagents for human IL-8 were purchased and used in assay development and validation. Assay development was completed in less than 3 days. The validated assay features a wide dynamic range (3 – 4,000 pg/mL), low minimum required dilution, short assay run time (60 minutes), and sensitivity (3 pg/mL). Quality control samples (QC's) run in human plasma were used to derive statistics that show the assay is precise and accurate with a total error of all QC levels of under 12%. Furthermore, beyond a luminometer with injectors, the SPARCL assay requires no specialized or capital equipment expense. The SPARCL technology enables rapid immunoassay development, delivers a biomarker assay with desirable performance characteristics and at the same time allows for considerable savings in labor, disposables and capital equipment in development, validation and in sample analysis compared to other immunoassay platforms. Existing software can be used for a SPARCL assay data acquisition, and as a result, there may not be a need to validate new software in existing Laboratory Information Management Systems (LIMS). The SPARCL assay is microtiter plate-based and is suitable for use with robotics and in higher throughput applications and may prove to be a valuable assay development and production tool.

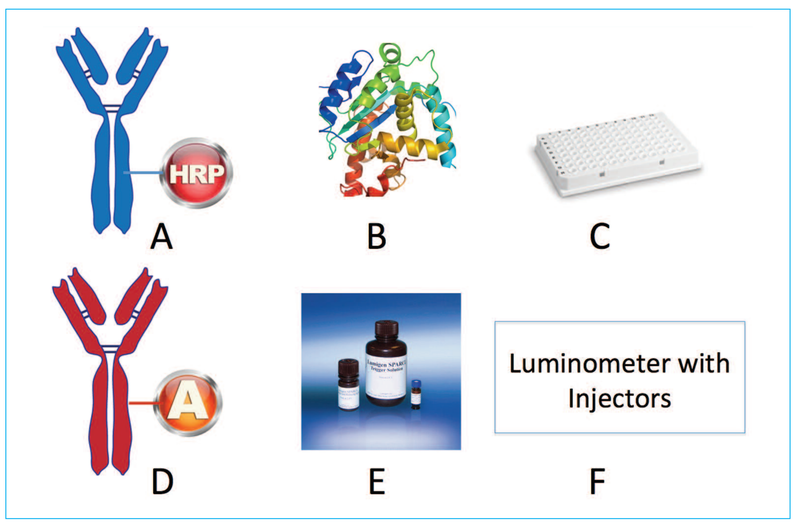


Figure 1. Key components to a SPARCL assay. **A -** HRP labeled Antibody. **B -** Immunoassay target (analyte). **C -** 96 well low binding white plate. **D -** Acridan labeled Antibody. **E -** SPARCL Kit. **F -** Luminometer.

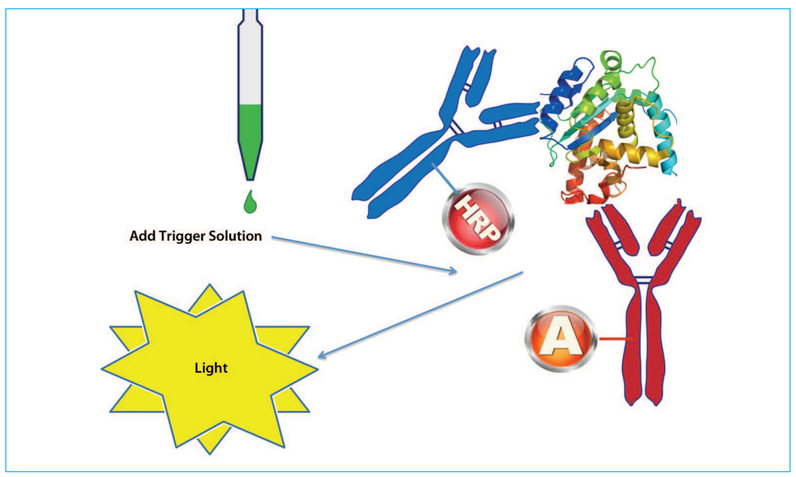


Figure 2. A representative SPARCL assay. Specific antibody and antigen bring acridan and HRP into close proximity. The addition of hydrogen peroxide based trigger solution interacts with acridan and HRP, causing a flash of light.

MATERIALS AND METHODS

Antibodies: A mouse anti-human IL-8 monoclonal antibody was purchased from R&D Systems (Minneapolis MN), catalog number MAB208 and was conjugated with acridan. A HRP-conjugated mouse anti-human IL-8 monoclonal antibody was purchased from Anogen, a division of YES Biotech Laboratories (Mississauga Ontario, Canada), catalog number MO-C40017T.

Calibrator: Recombinant human IL-8 was purchased from R&D Systems (Catalog Number 208-IL).

Matrix: Human plasma was obtained from Bioreclamation. The fetal calf serum and RPMI-1640 was obtained from Sigma.

SPARCL Kit: The SPARCL kit was obtained from Lumigen (Southfield, Michigan). The kit is composed of a hydrogen peroxide based trigger solution, a background reducing agent and acridan.

Acridan Labeling:
Add 500 µL of DMF to Lumigen SPARCL labeling reagent vial.
In a separate 1.5 mL microfuge tube mix the following:

- 708.7 µl of 0.05M sodium borate buffer, pH 8.5
- 41.3 µl of Lumigen SPARCL labeling reagent in DMF (from step 1)
- 250 µl of 1 mg/mL antibody (approximately 160 kDa)

Mix by inverting the tube 4-5 times, cover the tube with aluminum foil and let stand for 30 min. at room temperature. Place the labeling reaction tube on a rocker to mix at 2-8°C overnight.

Assay Buffer: PBS containing 0.1% BSA was used for dilution of the conjugated antibodies.

Assay Development: A fit-for-purpose approach was used for assay development and validation[1].

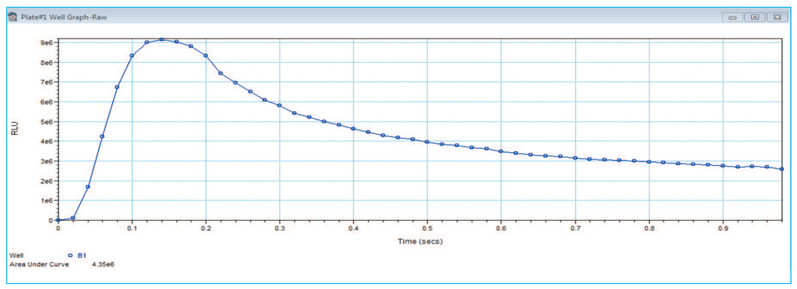
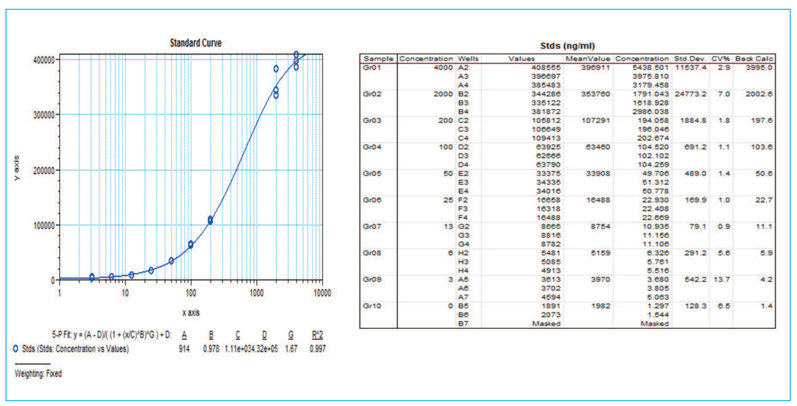


Figure 3. A typical SPARCL pattern of flash luminescence. Light energy was captured every 0.02 seconds for a total time of 1.0 second. Note peak luminescence at 0.15 seconds after trigger injection.



Stds (ng/ml)

Sample	Concentration	Wells	Values	MeanValue	Concentration	Std Dev.	CV%	Back Calc
Gr01	4000	A2	408555	396911	5438.501	11537.4	2.9	3995.0
		A3	396697		3975.810			
		A4	385483		3179.458			
Gr02	2000	B2	344286	353760	1791.043	24773.2	7.0	2002.6
		B3	335122		1618.928			
		B4	381872		2986.038			
Gr03	200	C2	105812	107291	194.058	1884.8	1.8	197.6
		C3	106549		196.046			
		C4	109413		202.674			
Gr04	100	D2	53925	53460	104.520	691.2	1.1	103.6
		D3	52666		102.102			
		D4	53790		104.259			
Gr05	50	E2	33375	33908	49.706	489.0	1.4	50.6
		E3	34336		51.312			
		E4	34016		50.778			
Gr06	25	F2	16658	16488	22.930	169.9	1.0	22.7
		F3	16318		22.408			
		F4	16488		22.669			
Gr07	13	G2	8665	8754	10.935	79.1	0.9	11.1
		G3	8816		11.156			
		G4	8782		11.106			
Gr08	6	H2	5481	5159	6.326	291.2	5.6	5.9
		H3	5085		5.761			
		H4	4913		5.516			
Gr09	3	A5	3613	3970	3.680	542.2	13.7	4.2
		A6	3702		3.805			
		A7	4594		5.053			
Gr10	0	B5	1891	1982	1.297	128.3	6.5	1.4
		B6	2073		1.544			
		B7	Masked		Masked			

Figure 4. Screen capture of Softmax Pro 5.4 data acquisition software showing typical standard curve. Note the wide dynamic range of 3 to 4000 pg/mL.

Characteristic	Statistic	QC 200	QC 100	QC 50	QC 25	QC 12.5	QC 6.25
# Results	Mean (N)	6	6	6	6	6	6
Accuracy	Mean Bias (%RE)	0.75	3.5	0.8	3.6	4.8	2.4
Precision	%CV	6.1	3.4	1.9	11.1	4.9	7.7
Total Error	%RE + %CV	6.9	6.9	2.7	14.7	9.7	10

Table 1. Inter assay precision and accuracy. QC's were made in neat matrix. Note that the total error ranged from 2.7 to 14.7 percent.

LUMIGEN SPARCL™ DETECTION KIT

A: Lumigen SPARCL Labeling Reagent
- Activated NHS ester of an acridan compound

B: Lumigen SPARCL BGR
- Background reducing agent used to enhance S/N ratios

C: Lumigen SPARCL Trigger Solution
- Hydrogen peroxide containing solution for signal generation

** Also included in the kit is a Borate Buffer Pack for the labeling reaction*

TYPICAL LUMIGEN SPARCL™ ASSAY WORKFLOW

Step 1: Label one of the antibody with the activated SPARCL label. No purification required.

Step 2: Add antibody solutions and sample; Incubate for 30 minute

Step 3: Add SPARCL BGR

Step 4: Read the luminescence signal by injecting SPARCL Trigger Solution on a luminescence plate reader

CONCLUSIONS

SPARCL allows for rapid assay development due to short assay run times, a homogenous assay format and flash luminescence.

The SPARCL assay has a wide dynamic range (2.5+ logs), is sensitive (LLOQ of 3 pg/mL) and has a low MRD (1:10).

SPARCL assays are cost effective in terms of capital equipment, labor, reagent usage, disposables, buffers and service contracts on equipment

The SPARCL technology is less complicated than other commonly used platforms while providing the performance of commonly used platforms.

Reference
1. Lee JW, Devanarayan V, Barrett YC, Weiner R, Allinson J, Fountain S, Keller S, Weinryb I, Green M, Duan L, Rogers JA, Millham R, O'Brien PJ, Sailstad J, Khan M, Ray C, Wagner JA. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006 Feb;23(2):312-28