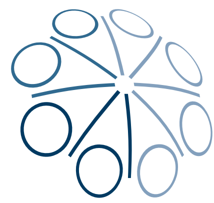
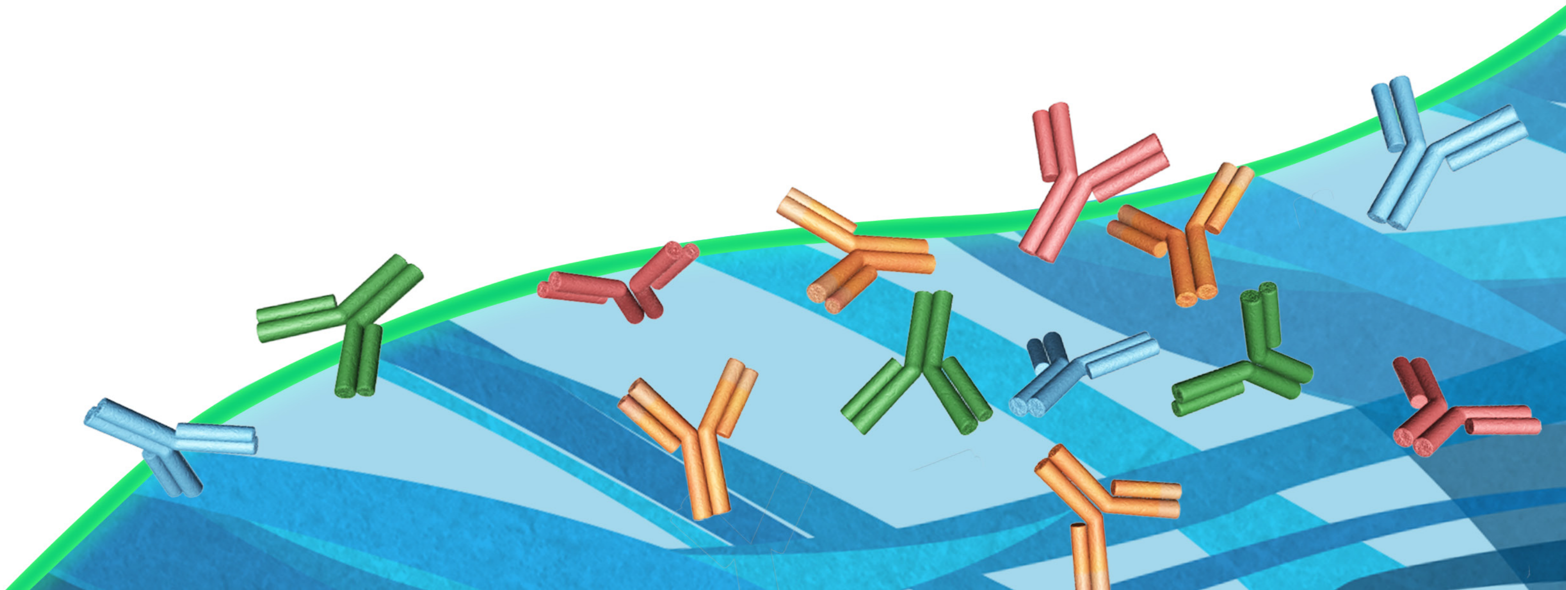


Genalyte

Novel Multiplex Measurement of Macromolecules Binding To A Solid Phase Using Photonic Microring Sensors

Applications For Autoimmunity



Introduction

Genalyte has developed a revolutionary multiplex detection technology based on silicon photonics that uses ring resonance to measure binding of macromolecules to sensors on a miniature silicon chip. The Maverick Detection System detects changes in resonance wavelength as macromolecules such as virus particles, proteins and nucleic acids bind to the sensors. An application for autoimmunity is the measurement of autoantibodies in serum.

Materials and Methods

Six antigens (SS-A/Ro 60, SS-B/La, Sm, RNP, Scl-70 and Jo-1) were spotted onto separate microring sensors on the silicon chip. Patient samples were diluted and run over the chip. Direct binding of macromolecules to the antigens is observed by the Maverick in 4 minutes (Figure 1). Quantification of specific IgG autoantibodies bound to each antigen is obtained by flowing anti-IgG over the chip and measuring the amount bound. The resulting change in resonance wavelength is expressed as Genalyte Response Units (GRU) and are the change in picometers. The assay takes less than 10 minutes.

Results

In a method comparison study to ELISAs with over 320 samples positive for one or more of the antigens the positive percent agreements ranged from 79% to 95%, with total agreement from 94% to 100% (Table 1). There was no evidence for cross-reactivity between antigens. Linear regression of values in ELISA compared to PRI show R2 values around 0.90 (Figure 2). Precision studies yielded percent CVs less than 3% for high positive samples and less than 8% for moderately positive samples (Table 2). The analytical measuring ranges of the assays are greater than 2 orders of magnitude (Figure 3). Percent recovery studies show linearity over the complete range. In a study with 128 normal donors, 5 of 6 assays yielded 100% specificity, and the other was greater than 99% specific (not shown).

CONCLUSIONS

This technology is most applicable to measuring autoantibodies when accuracy, multiplexing and quick turnaround time are of high importance.

TABLE 1. *Method comparison with ELISA*

Cutoff=20 PRU

	SS-A/Ro 60	ELISA +	ELISA -
PRI	+	54	1
	-	6	289

Total Sample = 350

Total Agreement	98.0%
Positive Agreement	90.0%
Negative Agreement	99.7%

Cutoff=20 PRU

	SS-B/La	ELISA +	ELISA -
PRI	+	26	5
	-	7	312

Total Sample = 350

Total Agreement	96.6%
Positive Agreement	78.8%
Negative Agreement	98.4%

Cutoff=20 PRU

	RNP	ELISA +	ELISA -
PRI	+	56	6
	-	9	278

Total Sample = 349

Total Agreement	95.7%
Positive Agreement	86.2%
Negative Agreement	97.9%

Cutoff=20 PRU

	Scl-70	ELISA +	ELISA -
PRI	+	21	7
	-	3	318

Total Sample = 349

Total Agreement	97.1%
Positive Agreement	87.5%
Negative Agreement	97.8%

Cutoff=20 PRU

	Sm	ELISA +	ELISA -
PRI	+	28	17
	-	3	301

Total Sample = 349

Total Agreement	94.3%
Positive Agreement	90.3%
Negative Agreement	94.7%

Cutoff=20 PRU

	Jo-1	ELISA +	ELISA -
PRI	+	18	0
	-	1	330

Total Sample = 349

Total Agreement	99.7%
Positive Agreement	94.7%
Negative Agreement	100.0%

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FIGURE 1. Sensorgrams of a negative sample and a positive samples showing the primary binding to the antigen in the first step, and amplification of bound IgG in the second step.

The GRU on the Y axis represent the picometer shift of the resonant wavelength as mass binds to the antigen above a specific probe over time. In the negative control only the anti-IgG spot is amplified, showing that both patient sample and amplification reagent were flowed over the chip, and thus the negative results are true negatives. The RNP and Sm positive sample shows amplification for only those 2 antigens, and the anti-IgG control.

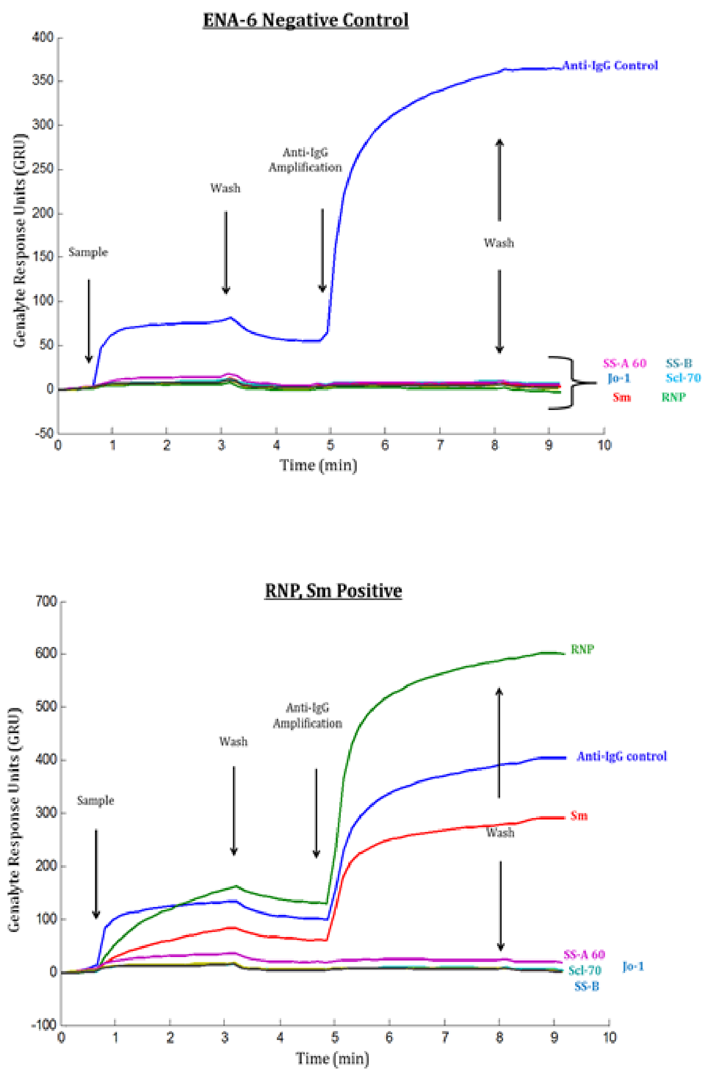

FIGURE 2. Representative positive samples and negative samples from the method comparison study of SS-A and RNP had their GRU normalized against the Units of ELISA, and then plotted against each other to show how values in GRU correlated with ELISA Units.

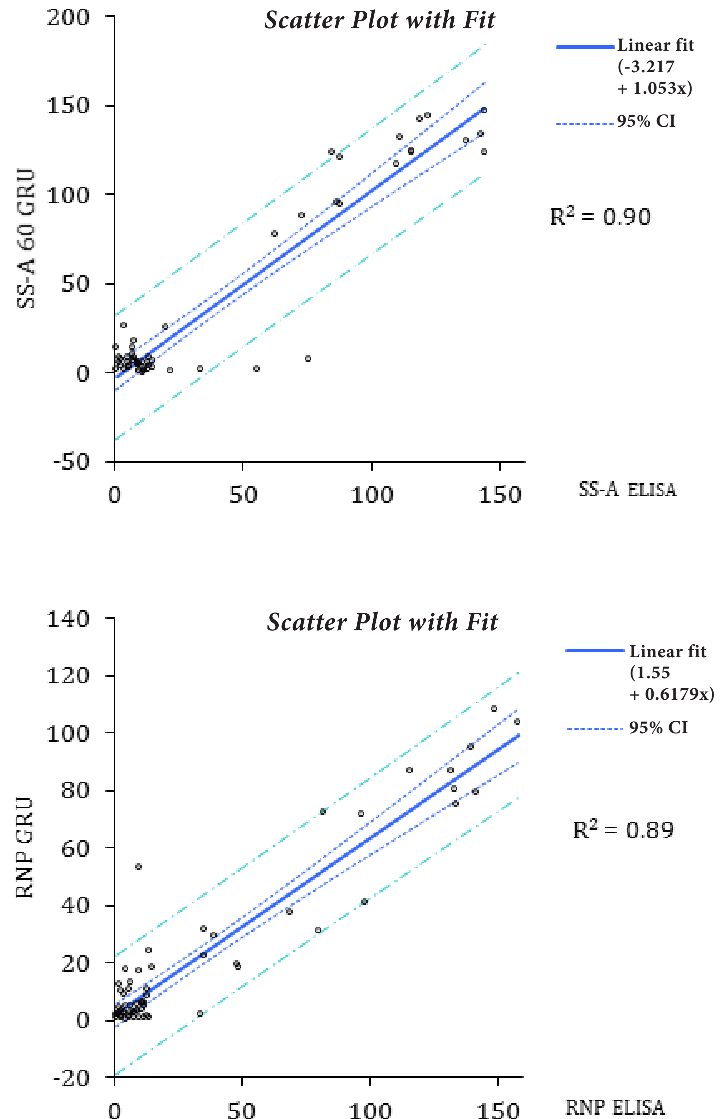

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TABLE 2. *Within assay precision was determined by running the same sample 12 times in one array.*

Within Assay Precision

RNP High Positive

	GRU	S.D.	%CV
Avg Channel 1	360	6	1.8%
Avg Channel 2	361	12	3.3%
Avg Total	360	10	2.6%

RNP Moderate Positive

	GRU	S.D.	%CV
Avg Channel 1	149	9	6.0%
Avg Channel 2	153	14	8.9%
Avg Total	151	12	7.6%

Jo-1 High Positive

	GRU	S.D.	%CV
Avg Channel 1	639	13	2.0%
Avg Channel 2	638	17	2.6%
Avg Total	638	15	2.3%

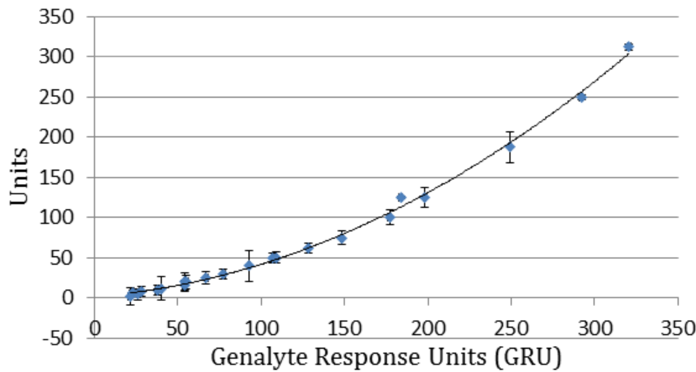
Jo-1 Moderate Positive

	GRU	S.D.	%CV
Avg Channel 1	149	6	4.1%
Avg Channel 2	153	10	6.9%
Avg Total	151	10	5.8%

FIGURE 3. *The analytical measuring ranges of RNP and Sm are shown in the large graphs where the GRU are plotted against the dilution factor (expressed as Units). The 3 smaller graphs under each large graph show that when the calculated value of a dilution is plotted against the measured value of the dilution for the top, middle and bottom of the analytical measuring range, the result has a linear fit with R^2>0.9, a slope of 1.0 +/- 0.1, and a Y intercept less than 10% of the highest point on the curve. Thus, over 2 orders of magnitude show linearity.*

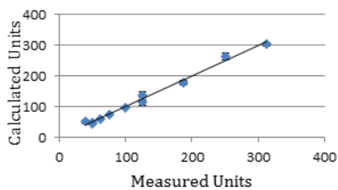
RNP Standard Curve

$y=0.0025x^2+0.1544x+1.9121$
$R^2=0.9972$



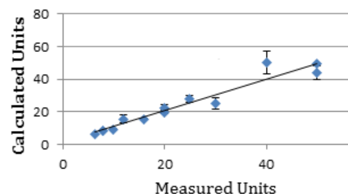
RNP Percent Recovery Top

$y=0.992x+0.462$
$R^2=0.9894$



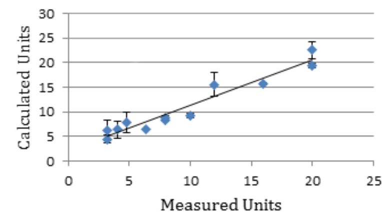
RNP Percent Recovery Middle

$y=0.9701x+1.2256$
$R^2=0.9362$



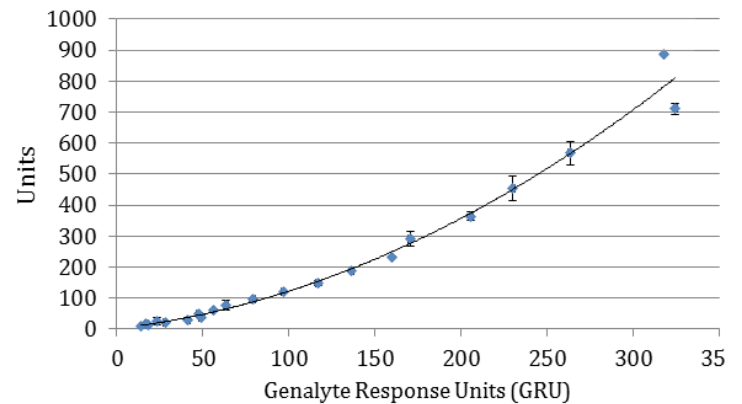
RNP Percent Recovery Bottom

$y=0.9244x+1.9987$
$R^2=0.9369$



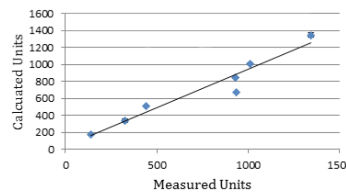
Sm Standard Curve

$y=0.0057x^2+0.6316x+1.9777$
$R^2=0.9824$



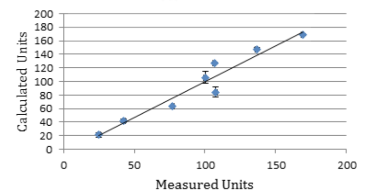
Sm Percent Recovery Top

$y=0.9017x+36.837$
$R^2=0.937$



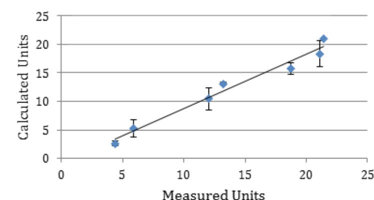
Sm Percent Recovery Middle

$y=1.0639x-6.6678$
$R^2=0.934$



Sm Percent Recovery Bottom

$y=0.9572x-0.8656$
$R^2=0.9741$



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