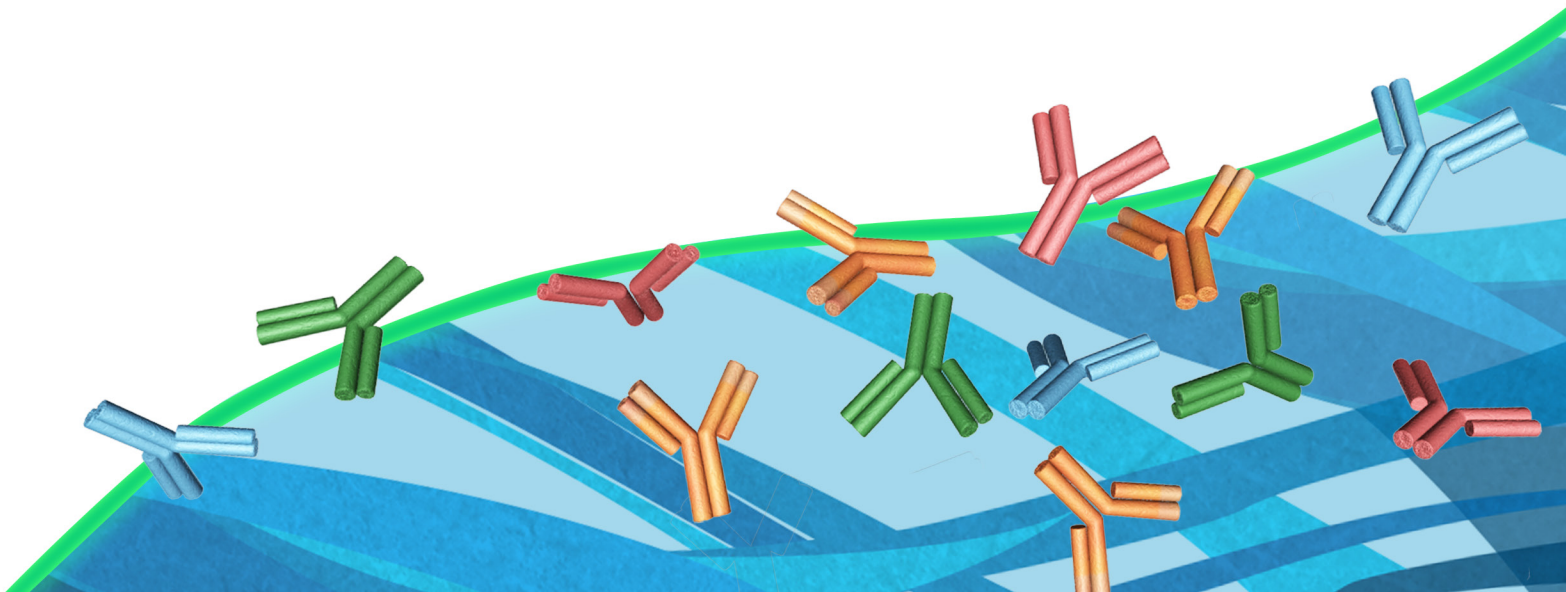
Genalyte

Detection of Anti-Drug Antibodies in Rabbits with a Multiplex Photonic Ring Immunoassay

The present study shows the performance characteristics of a Rabbit Multi-Tier Anti-Drug Antibody (rMT-ADA) assay. This is a multiplex assay that measures the isotype of anti-drug antibodies (ADA) in a single run. The sensitivity and free drug tolerance exceed FDA guidelines.



Introduction

There is currently exceptional growth in the development of biologic therapeutics. Along with their impressive clinical utility has come the realization that patients make an antibody response against many protein drugs. During development of a new drug, its antigenicity is tested in animal models, such as rabbits. We have developed a procedure to measure the isotype of ADA in rabbit serum.

Materials and Methods

All assays are run on the Maverick™ instrument, which is designed to measure binding of macro-molecules to ring sensors on small (3.5 by 5.6 mm) chips, based on the change in wavelength of ring resonance when a macromolecule binds to the surface of the chip above the ring. Each chip has 2 flow channels with 16 ring sensors per channel. In the Rabbit MT-ADA Photonic Ring Immunoassay (PRI), 4 sensors are each spotted with a specific capture probe: anti-Rabbit IgG (Fc), anti-rabbit IgM, anti-rabbit IgA, and streptavidin as positive control. Binding of ADA is detected by a shift in the wavelength of the ring resonance associated with that probe. The shift is proportional to the mass that has bound above the ring and is measured in Genalyte Response Units (GRU). All steps of the assay from running the samples over the chip, wash steps, and amplification of bound analyte are automatically performed by the Maverick™.

Specifically, with mouse IgG as the model drug, mouse IgG is biotinylated with a commercially available reagent. After off-line affinity capture and elution (ACE) of ADA performed in a 96 well plate, the eluted ADA are put into a 96 well plate, and both the plate and the rMT-ADA chip array are put into the Maverick instrument. All further steps are performed by the instrument. The purified ADA are flowed over the chip and any antibodies present are captured by the specific anti-isotype probes. Next, biotinylated mouse IgG is flowed over the chip, followed by streptavidin-phycoerythrin (SA-PE) for amplification of the biotinylated drug bound by the ADA. The amount of mass bound by each isotype capture probe is determined by the shift in the ring resonance of that probe. The SA-PE is used because of its large mass, not because of its fluorescent properties.

Results

The affinity capture and elution (ACE) technique (Figure 1) is used to purify ADA from the rest of the immunoglobulins in the serum. Using ACE we were able to detect the clinically relevant level of 250ng/ml ADA in the presence of 10 µg /mL free unlabeled drug (Table 1) at a signal to noise ration of greater than 10. With a cutoff of twice background, the lower limit of detection was 15.6ng/ml ADA in the presence of 10ug/ml free unlabeled drug (Figure 2).

FIGURE 1. *Outline of the Affinity Capture and Elution (ACE) procedure.*

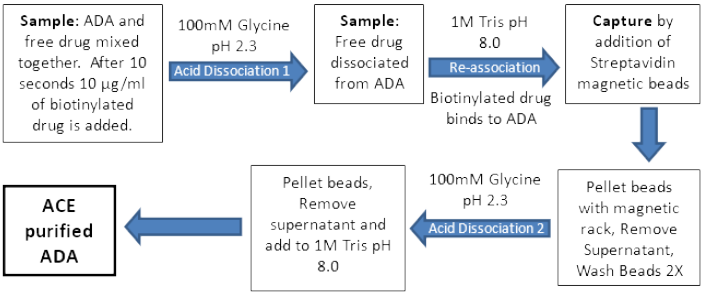


TABLE 1. *RAMADA with ACE at target sensitivity of 250ng/ml ADA in presence of 10µg/ml in 100% normal rabbit serum.*

Rabbit anti-mouse added to normal rabbit serum	Unlabeled mouse IgG	Detection	Anti-Rabbit IgG (GRU)	Signal to Noise Ratio
250 ng/mL 0	10 ug/ mL	SA-PE (0.1 mg/ml)	141 13	10.8

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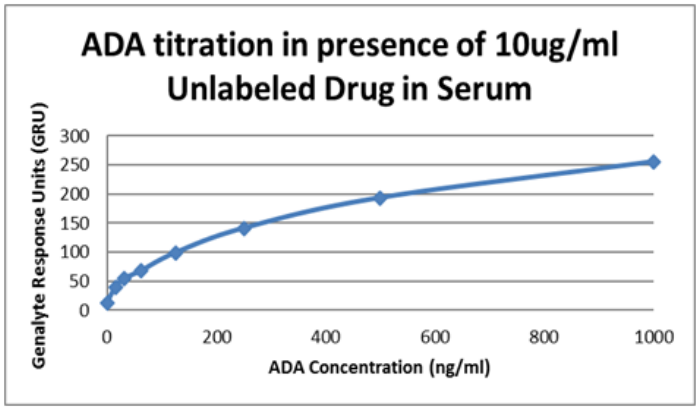
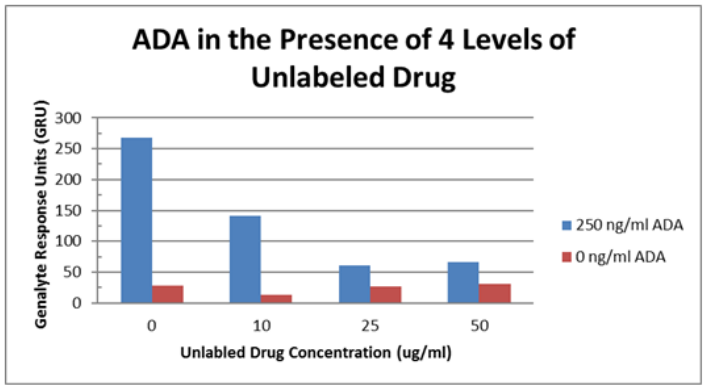
FIGURE 2. *With use of ACE procedure, the assay yielded sensitivity of 15.6 ng/mL ADA.*



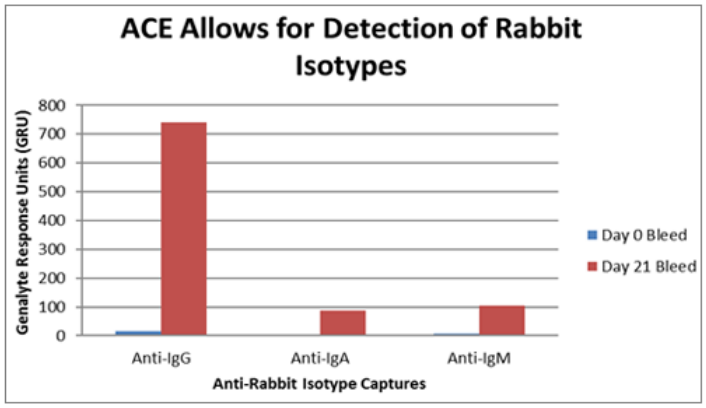
The free drug tolerance of rabbit anti-mouse IgG at 250 ng/mL in the rMT-ADA PRI was tested in serum in the presence of free drug at 50, 25, 10 and 0 ug/mL. The tests showed assay sensitivity was maintained at 250 ng/mL ADA in the presence of 50 µg/mL free unlabeled drug (Figure 3). The ACE procedure was performed at each concentration and the antibodies run in the Maverick™ instrument.

FIGURE 3. *Following the ACE procedure, assay maintained sensitivity at 250 ng/mL ADA in the presence of 50 µg/mL unlabeled drug.*

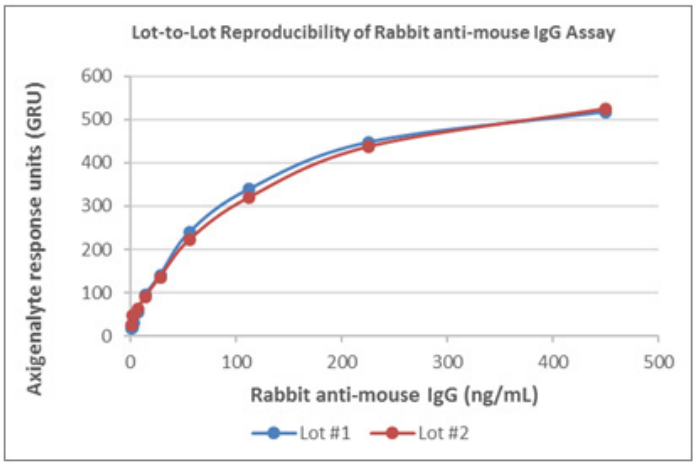


Isotyping of IgM, IgA, and IgG in immunized rabbits was demonstrated (Figure 4).

FIGURE 4. *A rabbit was immunized with mouse IgG. Bleeds (Day-0 and Day-21 post immunization) were assayed with the ACE workflow and run on the rMT-ADA.*



There was very good lot-to-lot reproducibility as shown in the graph below. Two lots of spotted chips and assay reagents were tested.



Within run precision at various concentrations of ADA was determined by running samples at the concentrations noted in the table below 12 times each on the same array. For all positive samples the %CV was less than 7%.

Within run precision N=12

Sample	GRU	S.D.	%CV
High Positive Sample	540	15	3%
Medium Positive Sample	295	13	4%
Low positive Sample	72	5	7%
Negative Control	13	6	50%

Between day precision at various concentrations of ADA was determined by running the same samples on 6 different days as shown in the table below. For all positive samples the %CV for between day precision was less than 9%.

Between day precision N=6

Sample	GRU	S.D.	%CV
High Positive Sample	525	17	3%
Medium Positive Sample	284	19	7%
Low positive Sample	75	7	9%

To determine the cutoff between positive and negative, sera from 18 normal rabbits was purchased and tested on the rMT-ADA. The results are in the table below.

Normal rabbit sera

N=18	Anti-IgG	Anti-IgA	Anti-IgM	Streptavidin Control Spot
Average	23	14	16	554
SD	7	4	6	15
Avg+2SD	37	22	28	583
Highest	46	17	20	563

Note: One rabbit blood sample had high pre-existing antibodies to mouse IgG, 260 GRU for IgG. This rabbit sample was notincluded in the data analysis.

Conclusions

Rabbit anti-mouse ADAs can be detected and isotyped at high sensitivity by biotinylating the drug (mouse IgG) with standard methods and using the ACE procedure to purify the ADA from serum. In the rMT-ADA PRI run on the Maverick, rabbit ADA isotypes were captured by the anti-rabbit IgM, IgA, and IgM antibodies on the chip. Without ACE, the presence of free drug in serum inhibits direct capture of the rabbit IgG ADA at 500ng/ml, twice above target sensitivity.

Target sensitivity of 250ng ADA in the presence of 10ug/ml unlabeled drug in serum was met using the ACE procedure. The target sensitivity is maintained in the presence of 50µg/ml unlabeled drug in 100% serum. Sensitivity of Rabbit IgG anti-mouse IgG is detected at levels as low as 15.6ng/ml in 100% serum in the presence of 10µg/ml of unlabeled drug.

In the model system, assay results met and exceeded sensitivity and free drug tolerance targets of 250 ng/mL in the presence of 25 µg/mL unlabeled drug. Thus, this assay can detect the isotypes and of anti-mouse IgG antibodies in a single run over the capture antibodies on the multiplex chip. The rMT-ADA PRI has high sensitivity for rabbit IgG, IgM, and IgA anti-drug antibodies, complete serum tolerance, and high free drug tolerance.

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