Genalyte

Detection of Isotype of Anti-Adalimumab Antibodies using a Multiplex Photonic Ring Immunoassay

The present study shows the performance characteristics of a human anti-Adalimumab Multi-Tier Anti-Drug Antibody (MT-ADA) assay. This is a multiplex assay that determines the isotype and IgG subclass of ADA in a single run, including IgG4. 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 produce an antibody response against many protein drugs. Regulatory agencies are now requiring evaluation of all new protein drugs for their immunogenicity, including isotype and subclass of ADA in some cases[1-3].

There is growing concern that methods used to detect ADA include the ability to measure IgG4. Since IgG4 has a unique structure caused by switching Fab domains to generate a bispecific antibody, some methods like the bridging assaydo not detect IgG4 ADA at the same sensitivity as other subclasses. The Genalyte hMT-ADA detects all isotypes and IgG subclasses with high sensitivity.

Materials and Methods

All experiments were conducted using the Genalyte hMT-ADA assay and run on the Maverick™ M24 instrument, which is designed to measure binding of macro-molecules to ring sensors on small (3.5 by 5.6mm) chips. In the hMT-ADA PRI (Photonic Ring Immunoassay), seperate sensors are spotted with an anti-isotype capture probe to the Fc portion of human IgA, IgM, IgE, IgG1, IgG2, IgG3 or IgG4 and astreptavidin control. Binding of ADA is detected by a shift in the wavelength of 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™ M24.

Specifically, Adalimumab is biotinylated with a commercially available reagent. After off-line affinity capture and elution (ACE) of ADA performed in a 96 well plate, the ADA are flowed over the chip, and any ADA present are captured by the specific anti-isotype/subclass probes. In the next step, biotinylated Adalimumab is flowed over the chip, followed by amplification with streptavidin coated nanospheres.

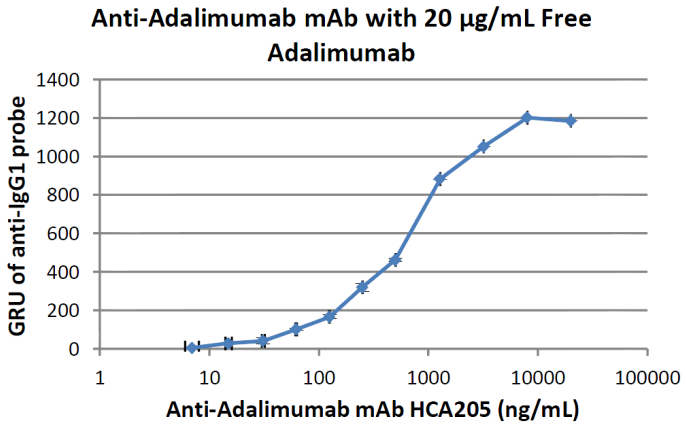
The amount of mass bound by each isotype and IgG subclass capture probe is determined by the shift in the ring resonance of that probe.

Results

Monoclonal anti-Adalimumab IgG1 (HCA205) from AbD Serotec, was spiked into humanserum at different dilutions. The monoclonal spiked sera were treated using the ACE procedure and run on the hMT-ADA assay on the Maverick™.

The sensitivity of the assay in serum in the absence of free drug is 16 ng/ml, which is greater than twice background (not shown).

The sensitivity of the assay in serum in the presence of 20 µg/ml of free Adalimumab is 62 ng/ml of ADA. Note that the graph below is in log scale and the initial concentration is 20,000 ng/ml of ADA.



The free drug tolerance of monoclonal anti-Adalimumab HCA205 at 250 ng/ml in the MT-ADA was tested in serum in the presence of free drug at 40, 20, 10, 5 and 0 µg/ml. The ACE procedure was performed at each concentration and the antibodies run in the Maverick™ instrument.

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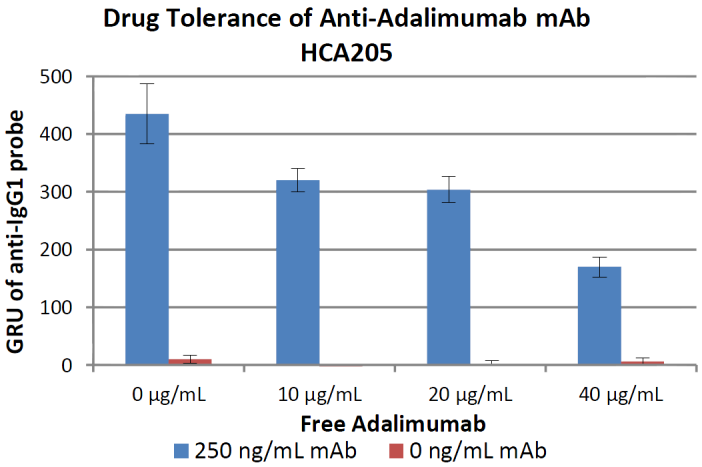

Within run precision at various concentrations of ADA was determined by running samples at the concentrations noted in Table 1 below 6 times each on the same array. For all positive samples the %CV was less than 14%.

TABLE 1. *Within run precision N=6*

1000 ng/ml		250 ng/ml		62.5 ng/ml		0 ng/ml	
GRU	1137	GRU	741	GRU	364	GRU	36
S.D.	33	S.D.	64	S.D.	52	S.D.	7
%CV	3%	%CV	9%	%CV	14%	%CV	19%

Between day precision at various concentrations of ADA was determined by running the same samples on 8 different days as shown in Table 2 below. For all positive samples the %CV for between day precision was less than 20%.

TABLE 2. *Between day precision N=8*

1000 ng/ml		250 ng/ml		62.5 ng/ml		0 ng/ml	
GRU	1107	GRU	749	GRU	349	GRU	30
S.D.	117	S.D.	100	S.D.	69	S.D.	16
%CV	11%	%CV	13%	%CV	20%	%CV	54%

Sera from eleven patients with rheumatoid arthritis (RA) or Crohn's disease who had taken Adalimumab were purchased from Bioreclamation. Their ADA status was not-known. They were tested on the human MT-ADA assay. Results are shown in Table 3 below.

TABLE 3. *Patients taking Adalimumab*

Disease	Lot #	Anti-IgG4	Anti-IgG3	Anti-IgG2	Anti-IgG1	Anti-IgM	Anti-IgA	Anti-IgM
RA	1	<5	13	<5	<5	<5	<5	<5
	2	<5	8	<5	7	24	43	<5
	3	441	47	<5	52	<5	<5	<5
	4	<5	15	<5	156	13	45	<5
	5	158	212	7	328	<5	12	6
	6	<5	6	<5	<5	<5	<5	5
	7	519	392	29	594	<5	39	<5
	8	37	22	<5	56	<5	<5	<5
	9	35	159	<5	382	<5	39	<5
Crohn's	10	123	41	<5	226	<5	7	<5
	11	37	164	<5	376	<5	<5	5
*Cuttoff		40	40	40	40	100	200	100

*For the purpose of this study, the cutoff was calculated by testing 23 normal samples and then using the average GRU + 2 standard deviations for each isotype.

Eight of the 11 (72%) patients taking Adalimumab were ADA positive on the MT-ADA PRI. Interestingly, in sample 3, IgG4 is the predominant subclass.

Five samples from patients taking Adalimumab who were tested on the LISA Tracker Theradiag ADA ELISA to measure anti-Adalimumab antibodies were also tested on the Maverick™ hMT-ADA PRI. Two of the samples tested positive on both the Theradiag ELISA and MT-ADA as shown in Table 4. The remaining three samples were negative on both assays.

TABLE 4. *Patients tested by other technologies*

Sample ID	Anti-IgG4	Anti-IgG3	Anti-IgG2	Anti-IgG1	Anti-IgM	Anti-IgA	Anti-IgE
1	<5	<5	<5	18	38	11	5
2	<5	5	<5	18	<5	<5	6
3	1232	402	202	451	29	114	25
4	1226	684	562	928	85	231	52
5	<5	<5	<5	13	<5	6	8
Cutoff	40	40	40	40	100	200	100

Frequency of Isotype and IgG subclasses of ADA

As seen in Table 5 below, in the 10 patients who are positive for ADA to Adalimumab, the most common ADA are found in IgG subclasses IgG1, IgG3 and IgG4 at 100%, 80% and 60%,

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respectively. IgG2 and IgA are less common at 20%, while IgM and IgE have no ADA.

TABLE 5. *Frequency of Isotype and Subclasses of ADA*

N=10	Anti-IgG4	Anti-IgG3	Anti-IgG2	Anti-IgG1	Anti-IgM	Anti-IgA	Anti-IgE
Number	6	8	2	10	0	2	0
Frequency	60%	80%	20%	100%	0%	20%	0%

Conclusions

The sensitivity and free drug tolerance of the Maverick human MT-ADA PRI as applied to Adalimumab exceed the FDA guidelines of 250 ng/ml of ADA in the presence of 20 µg/ml of free drug. There is very high concordance between the results obtained with the Maverick MT-ADA PRI and other technologies.

There is increased pressure from regulatory agencies to ensure that ADA testing detects all isotypes and IgG subclasses.The ability to measure the isotypes and IgG subclasses of ADA in a single multiplex assay is an important improvement over other technologies.

Interestingly, there was one patient taking Adalimumab who was not tested by other technologies, but was predominantly IgG4 positive.

References

1.Guidance for Industry: Immunogenicity Assessment for Therapeutic Protein Products. www.fda.gov.

2. Chamberlain PD. Multidisciplinary approach to evaluating immunogenicity of biosimilars: lessons learnt and open questions based on 10 years' experience of the European Union regulatory pathway. Biosimilars 2014:4 23–43.

3. Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product. www.fda.gov.

Ordering Information

Part No.	Description
85000	Maverick M24 Detection System
85023	Maverick hMT-ADA PRI Kit

Please inquire for other species

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