

Multiplexed immunoassay approach to characterize antidrug antibody like specific reactivity

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Aim: Characterization of antidrug antibody (ADA) like reactivity has emerged as critical element of bio-analytical design and assessment of compound immunogenicity risk. **Materials & methods:** Multiplex immunoassay was applied to detect and characterize ADA like reactivity using Photonic Ring Immunoassay platform (Genalyte). Specific binding to human IgE or human recombinant IL21-receptor-Fc fusion using exogenous reagents as surrogates for drug specific reactivity was investigated. **Results:** Multiplexed assay format allowed identification of spiked antihuman IgE reactivity as murine IgG1 and endogenous antihuman recombinant IL21-receptor-Fc reactivity in rheumatoid arthritis sera as antihuman Fc-specific binding. **Conclusion:** The ability of a multiplex immunoassay platform to identify isotype and domain specificity of antidrug immunoglobulins was shown to be effective and should be considered when screening and characterizing pre- and postdose ADA reactivity.

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Assessment and evaluation of immunogenicity risk is an integral part of biotherapeutic drug development. Analysis often includes detection and characterization of drug-specific immunoglobulins in pre- and post-treatment samples [1]. For example, information obtained in predose treatment naive samples is critical as it is used during development of fit for purpose bioanalytical methods, including methods designed to detect postdose induction of drug-specific immune response [2]. Predose samples may contain drug binding matrix components that may or may not be immunoglobulins by nature, also referred to as preexisting reactivity [3,4]. Preexisting drug-specific antibodies have been discussed as a part of the overall drug immunogenicity risk potential [4,5]. Detection and appropriate characterization of preexisting reactivity, including detection of any preexisting drug-specific antibodies, is therefore important. Similarly, a postdose immunogenicity responses are typically assessed in a tier-based approach that includes initial sample screening followed by detailed analysis of antidrug antibodies (ADA) for the evidence of neutralizing activity, potentially isotype and drug domain-binding specificity [6,7]. Typically, domain-binding specificity is determined in a binding inhibition experiment, similar to specificity confirmatory analyses [8,9]. These methods may be complex and time or resource intensive.

Access to a robust-automated platform allowing detection and characterization of drug-specific reactivity in a multiplexed format would be particularly useful. Herein, we report on a feasibility assessment of the Maverick

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Photonic Ring Immunoassay (PRI) methodology based on analysis of reactivity against two model proteins: human IgE immunoglobulin and human recombinant IL21 receptor Fc (rhIL21R-Fc) fusion protein. A detailed description of Maverick technology has been previously published [10,11]. Briefly, the Maverick Detection System and multiplex PRI offer the ability to detect and characterize ligand-binding interactions in a multiplexed assay. The technology is based on individually addressable 30- μ m Photonic Microring Sensor™ clusters fabricated on the surface of a single-use silicon chip. 32 sensor clusters (four sensors per cluster) are arranged in two independent fluidic flow channels on each chip. Each sensor cluster is functionalized with a probe molecule such as a drug protein, antigen or a capture antibody of interest. Binding of proteins or antibodies to the probe molecule is monitored in real-time by measuring the magnitude shift in resonant wavelength of each sensor. This shift is directly proportional to the mass of the bound material, enabling detection of the target protein species [11]. In the Maverick MT-ADA assay kit, the sensor clusters are individually functionalized with antimouse, antirabbit or antihuman immunoglobulin class and subclass-specific capture antibodies. These capture antibodies bind target antibodies present in the sample and are then confirmed as ADA by subsequent binding of biotinylated drug and streptavidin-coated amplification bead complexes [10,11]. The MT-ADA chips were used in the present investigation in order to simultaneously detect and characterize drug-specific reactivity in naive human samples.

A multiplexed format immunoassay based on the Maverick PRI is described as an example of system to detect and characterize exogenously introduced murine antihuman IgE reagent and exogenous as well as endogenous reactivity to the hIL21R-Fc as assessed in human serum samples. Several other multiplex platforms are available [12,13]. The assessments described herein consisted of evaluating of multiplexed immunoassay platform for its ability to detect unknown reactivity and characterize the same by determining immunoglobulin isotype and specificity of binding. While examples presented herein are based on application of the Maverick-based platform, the main focus of the paper is to demonstrate the benefits of multiplexed immunoassay format during detecting and characterization of ADA like reactivity in unknown sample. ADA responses are known for lack of linearity and parallelism between individual samples due to a variety of ADA isotype, affinity of binding and epitope specificity. Therefore, it is common to apply semiquantitative approach to assay quality evaluation, for example, by including assay controls. The aim of the manuscript is to understand whether the multiplexing approach can provide a qualitative (i.e., yes/no) answer to the detection and characterization of ADA like response. Because of the semiquantitative or qualitative nature of the ADA methods, a limited assessment of the methodology was conducted and is described herein while avoiding a detailed qualification typical for a quantitative analytical protocol.

Materials & methods

Samples analyzed for endogenous & exogenous reactivity using Maverick detection system

Individual serum samples from treatment naive systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and normal human serum (NHS) donors were obtained commercially (Bioreclamation LLC, NY, USA). 25 drug naive NHS samples were spiked with monoclonal murine antibody specific to human IgE immunoglobulin (Abcam, MA, USA, cat. # ab106494) at concentrations ranging from 0.012 to 12.5 μ g/ml in 100% human serum. In a separate experiment, drug naive RA (n = 25) and SLE (n = 25) serum samples were spiked with human IL21 (EBioscience, CA, USA, cat. # 34-8219-85) at concentrations ranging from 0.012 to 12.5 μ g/ml in 100% human serum.

Biotin labeling of human IgE & rhIL21R-Fc

Human IgE (Abcam, cat. # ab152001) and rhIL21R-Fc (R&D Systems, MN, USA, cat. # 991-R2-100) at 0.4 mg/ml were biotinylated at a molar coupling ratio of 1:15 using the Thermo EZ-Link Sulfo NHS-LC biotinylation kit (Thermo Scientific, MA, USA) per manufacturer's recommendations [14]. Excess biotin was removed by buffer exchange into phosphate-buffered saline (PBS) + 0.5% NaN₃ using Zeba Pak columns (Thermo Scientific) per manufacturer's recommendation [15].

Acid treatment of samples & affinity capture elution for Maverick detection system

To improve specificity and sensitivity of the method, samples were initially pretreated using an Affinity Capture Elution (ACE) method. In the method, one part of biotinylated IgE or biotinylated rhIL21R-Fc at 0.2 mg/ml was added to four parts of serum samples. Samples were then treated with 0.1 M glycine buffer at pH 2.3 for 1 min before neutralization with 1 M Tris buffer at pH 8.5 at a 9:1 ratio. The samples were then incubated for 30 min at room temperature with shaking before the addition of 100 μ l (1 mg) of streptavidin-coated T1 streptavidin

(SA) magnetic beads (Life Technologies, CA, USA) diluted to 15% of stock in PBST to capture the biotinylated protein-binding protein complexes. The SA magnetic beads were used for amplification required to improve assay sensitivity. Each sample tube was placed into a magnetic rack for 4 min to pellet the beads. The supernatant was removed and discarded. The beads were washed by adding 1 ml PBST, vortexed, briefly centrifuged and placed back on the magnetic rack for 4 min. The supernatant was removed and discarded, the wash procedure was repeated for a total of three-times. After discarding the supernatant from the third wash, a second acid dissociation was performed to release any protein-bound material. For that, 45 μ l of 0.1 M glycine buffer at pH 2.3 was added to each sample tube. Beads were pelleted on the magnetic rack for 30 s and the supernatant was collected into new sample tubes containing 5 μ l of 1 M Tris buffer at pH 8.5 for neutralization.

Assessment of human IgE-specific reactivity

Analysis for exogenously introduced IgE-specific reactivity was performed by combining each ACE-treated sample (see above for the procedure) with biotinylated-IgE (final concentration of 1 μ g/ml). Samples were incubated for 30 min at room temperature on a shaker and assayed on the Maverick Mouse MT-ADA chip at a flow rate of 10 μ l/min for 5 min, followed by injection of 3% 0.1 μ M SA mag-beads (Ademtech, Pessac, France) at a flow rate of 10 μ l/min for 5 min. The Maverick MT-ADA biosensors used in the test were functionalized with antimouse Ig, IgG1, IgG2a, IgG2b, IgG3, IgM and IgE capture antibodies. PBST was used as a wash buffer between the assay steps and to generate baseline signal value. Relative amount of IgE-specific reactivity present in a sample was reported in Genalyte Response Units (GRU) determined by measuring the change in the resonant wavelength due to mass accumulation on the microring sensor relative to the baseline signal measured prior to the amplification step with the 0.1 μ M SA mag-beads (Ademtech). Sensor performance was controlled using serum samples unspiked or spiked with the murine antihuman IgE reagent (Abcam, final concentration: 2500 ng/ml) as negative and positive controls, respectively. For all assessments, a sample was defined as positive if the GRU values were two-times (cutoff) the level of the GRUs obtained at the background level.

The cutoff-based approach is most common for ADA detection assays. Typically, a cutoff (also called cut-point) value is defined based on performance of naive samples and will differentiate noise versus true positive signal. For example, if a sample generates a positive signal above the assay cut-point, it will be deemed ADA positive. The cutoff approach is commonly preferred over the quantitative ADA concentration determination method due to a variable nature of ADA response, including ADA affinity, epitope of binding and immunoglobulin isotype overall leading to a lack of signal parallelism between samples and lack of true ADA reference material.

Assessment of rhIL21R-Fc-specific reactivity

PRI for detection of rhIL21R-Fc-specific reactivity in RA & SLE disease serum samples

Individual RA and SLE serum samples were ACE treated (see above for the procedure) and flowed over the PRI chips functionalized with 250 μ g/ml of biotinylated IL21R-Fc at 10 μ l/min for 5 min. PBST was used as a wash buffer between the assay steps and to generate baseline signal value. Serum sample without or spiked with the rhIL21 reagent (final concentration: 2500 ng/ml, R&D Systems) were used as negative and positive controls, respectively. After initial assessments of the sensor performance using the positive and negative controls, 48 unknown samples were diluted 1:1 in PBST and screened in the assay.

Domain specificity characterization of the endogenous rhIL21R-Fc-specific reactivity in RA & SLE samples

To perform domain specificity characterization of the reactivity against IL21R-Fc, custom chips were manufactured by Genalyte. Specifically, flow channel 1 contained the following proteins immobilized on sensors: rhIL21R-Fc fusion, rhEphA1-Fc (R&D Systems, cat. #7146-A1-100), rhMAdCAM-1-Fc (R&D Systems, cat. #6056-MC-050) and rhIgG1-Fc (R&D Systems, cat. #110-HG-100). All reagents contained identical human Fc domains. Flow channel 2 contained sensors functionalized with capture reagents specific for human immunoglobulin isotypes IgG, IgM, IgA and IgE as well as IgG subclasses (IgG₁, IgG₂, IgG₃, IgG₄). Six RA and six SLE samples were ACE treated as per the above procedure and tested on the custom multiplex chip by flowing at a 10 μ l/min flow rate for 5 min over the two channels of the chip.

Isotype analysis of the endogenous rhIL21R-Fc-specific reactivity in RA & SLE samples

Maverick human MT-ADA kit containing reagents specific to human antibody isotypes IgG, IgM, IgA, IgE, IgG subclasses (IgG₁, IgG₂, IgG₃, IgG₄), and a streptavidin control sensor was used. Streptavidin-coated polystyrene

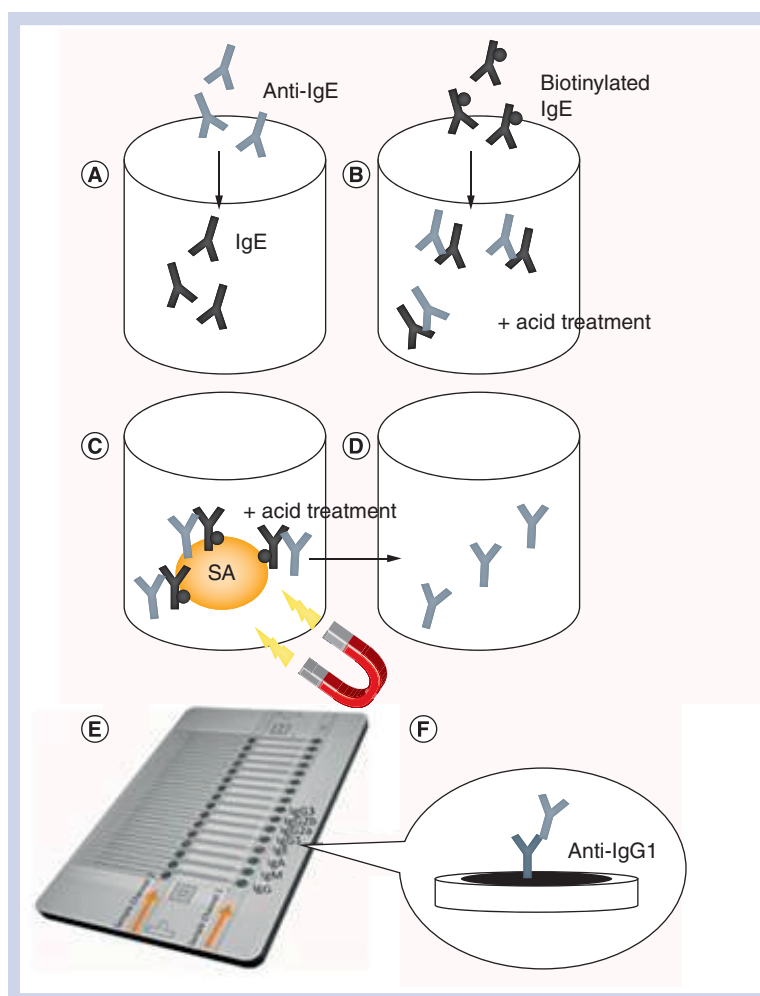


Figure 1. Summary of experimental procedure for isotyping of the anti-IgE-binding activity. (A) Anti-IgE was spiked into normal human serum and allowed to bind to IgE. An Affinity Capture Elution method was used to recover the spiked anti-IgE for the detection the Photonic Ring Immunoassay method. **(B)** Excess biotinylated IgE was added to the sample, followed by acid treatment of the sample. **(C)** After allowing the biotinylated IgE to bind to the spiked anti-IgE, the complexes were pulled down using magnetic streptavidin beads. **(D)** Bead-bound complexes were acid treated and the eluate recovered to yield anti-IgE. **(E)** The purified anti-IgE was flowed over a Maverick Mouse MT-antidrug antibody chip. **(F)** The anti-IgE was murine IgG1 in nature and captured on the corresponding anti-IgG1 sensor on the chip.

magnetic beads (0.1 μM SA mag-beads; Ademtech) were used as a secondary reagent to amplify the signal of the captured biotinylated drugs.

ACE treated RA and SLE samples were supplemented with the biotinylated rhIL21R-Fc reagent at 5 $\mu\text{g}/\text{ml}$ and flowed over the modified human MT-ADA chip at a flow rate of 10 $\mu\text{l}/\text{min}$ for 5 min, which was followed by 3% 0.1 μM SA mag-beads (Ademtech) at a flow rate of 10 $\mu\text{l}/\text{min}$ for 5 min. PBST was used a wash buffer between the assay steps and to generate baseline signal. For all assessments, a sample was defined as positive if the GRU values were two-times the level of the GRUs obtained at the background level.

Results

Screening & characterization of binding reactivity specific to human IgE immunoglobulin in NHS

NHS samples were spiked with murine antihuman IgE antibodies reagent with a known mouse IgG1 isotype at concentrations varying between 0.012 and 12.5 $\mu\text{g}/\text{ml}$. The samples were randomized and blinded prior to the analysis. The nature of the target protein (human IgE) was disclosed prior to the analysis. Spiked NHS samples were initially subjected to an ACE procedure, then incubated with biotinylated-human IgE immunoglobulin and flowed over the Maverick Mouse MT-ADA photonic biosensors sensors that was functionalized with antimouse Ig (total), IgG1, IgG2a, IgG2b, IgG3, IgM and IgE capture antibodies. Figure 1 summarizes the overall experimental procedure. ACE like procedures have been broadly described elsewhere and are typically applied to pretreat samples in order to purify drug-specific ADA by removing other potentially interfering factors, such as residual drug or other nonspecific human immunoglobulins [16]. Several controls containing murine antihuman IgE reagent were included to monitor system performance (see Methods section for the details).

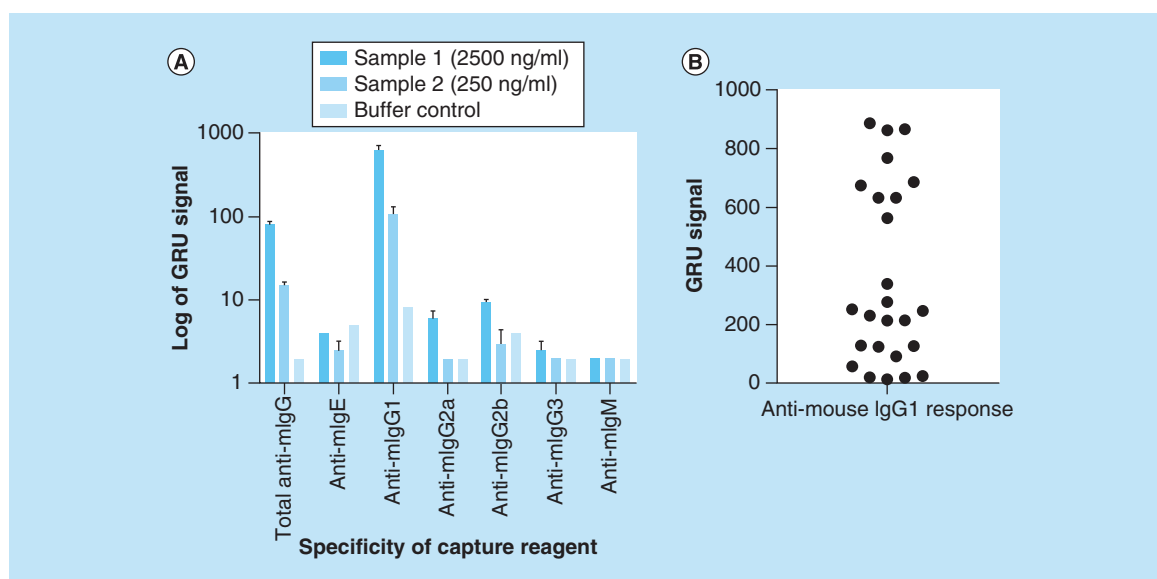
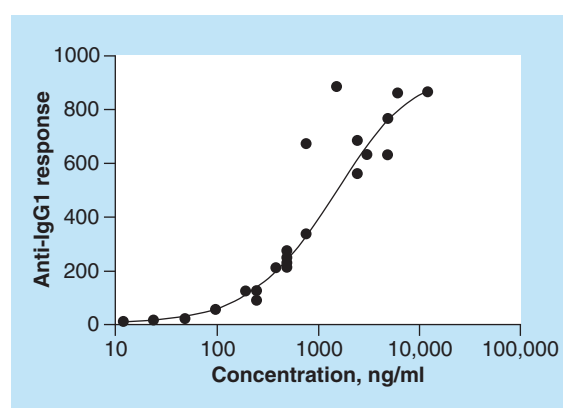


Figure 2. Maverick photonic ring immunoassay detects presence of murine IgG1 antihuman IgE-specific immunoglobulin activity in normal human samples. Murine IgG1 reagent was exogenously introduced into individual human samples at various concentrations. Representative sample (spiked with 2500 and 250 ng/ml) and control-generated results generated on sensors with various antimurine immunoglobulin isotype specificity are shown in (A). Distribution of the signal generated on the murine IgG1 sensor across all samples is shown in (B).

Figure 3. Maverick genalyte response unit signal proportionality versus concentration of the exogenous murine antihuman IgE reagent introduced into normal human serum samples at various concentration levels. Signal intensity (genalyte response unit) and concentration of murine antihuman IgE reagent (ng/ml) are shown.



All NHS samples containing murine antihuman IgE antibody were correctly identified in the test. Total antimouse IgG and mouse IgG1 isotype channels produced noticeably higher signal versus other subclass channels, therefore correctly identifying the nature of the interfering component as mouse IgG1 immunoglobulin with antihuman IgE specificity. In Figure 2A, representative sample performance is shown for samples containing 2500 ng/ml (high reactivity sample) and 250 ng/ml (low reactivity sample). Signal distribution generated by all NHS samples spiked with various concentrations of the exogenous mouse antihuman IgE reagent is shown in Figure 2B. Assay signal intensity was shown to increase proportionally to the concentration of the murine antihuman IgE antibody spiked into the sample (Figure 3). While the low limit of assay sensitivity was not directly evaluated, based on the 4PL fit of the dose-response curve, the assay detected murine antihuman IgE antibody at as low as 100 ng/ml. Common regulatory expectations for screening and confirmatory tiers of ADA assessment include ability of the assay to detect 100 ng/ml of ADA-positive control or less. Such sensitivity expectation is not extended to the characterization phase of ADA response, including isotype and domain-mapping evaluation. The multiplexing methodology presented herein should not be viewed as a primary method of detection of ADAs in human matrix but as a protocol applied as a later tier of ADA response characterization.

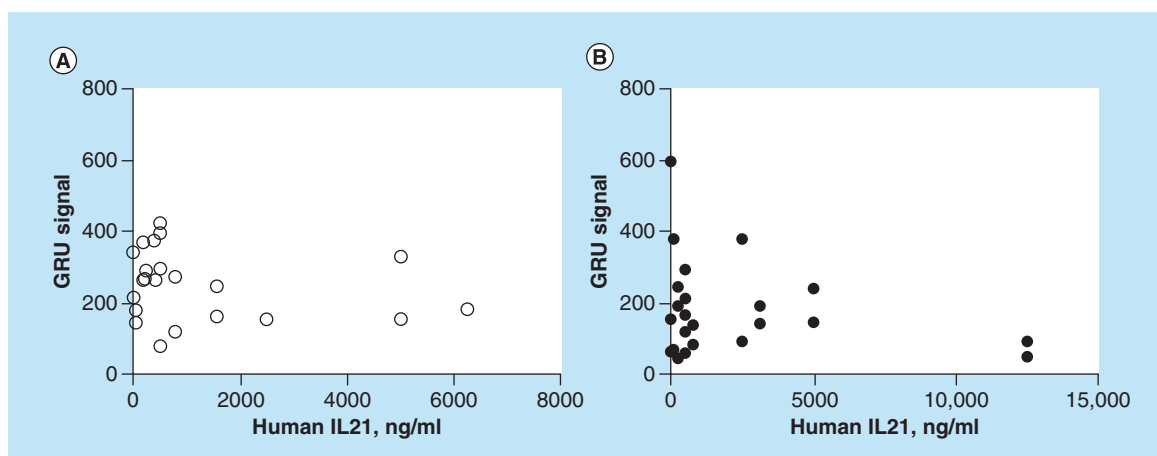


Figure 4. Dose response of the Maverick photonic biosensor functionalized with human recombinant IL21-receptor-Fc fusion protein versus concentration of exogenously spiked human IL21 ligand. Individual human serum samples were spiked at concentrations ranging from 12 to 12,500 ng/ml of human IL21. Signal intensity (GRU) and concentration of exogenously introduced IL21 ligand (ng/ml) show no correlation in both rheumatoid arthritis (A) and systemic lupus erythematosus (B) samples, suggesting presence of an unknown endogenous human rhIL21R-Fc fusion protein-specific reactivity. GRU: Genalyte response unit.

Screening of binding reactivity against the human IL21R-Fc fusion via a direct binding PRI: detection of 'true' endogenous reactivity in SLE samples

Binding experiments aiming to identify human IL21R-Fc fusion protein-specific reactivity in individual RA (n = 25) and SLE (n = 25) samples were performed using custom Maverick chips functionalized with an unlabeled rhIL21R-Fc. Samples were spiked with the rhIL21R-Fc-specific ligand (human IL21) at concentrations varying between 0.012 and 12.5 µg/ml. The samples were randomized and blinded prior to the analysis.

Variable signals were observed in the experiment indicating the presence of an unknown endogenous rhIL21R-Fc-specific reactivity (Figure 4). Similar performance was observed for both RA and SLE sample types (Figure 4A & B, respectively). In addition, the relative signal produced by the unknown endogenous reactivity in the samples was significant and independent of the concentration of the spiked IL21 ligand. Overall, it was concluded that an unknown endogenous reactivity specific to the human rhIL21R-Fc protein was detected by the Maverick PRI system in the RA and SLE sera.

Reagent carryover produced because of the elution step, a part of the ACE sample pretreatment procedure, was considered as a possible cause for the observed signal level and variability. Although such carryover may confound results of the test, the specific nature of anti-immunoglobulin isotype capture antibody reagents was expected to prevent such negative impact. Additional source of variability may be due to the suboptimal reproducibility of the ACE procedure extraction step. Yet, it should be noted that analysis of samples there were not spiked with the IL21 material also produced measurable anti-rhIL21-R signal. To characterize this unknown reactivity, RA and SLE sera were assessed using a custom Maverick human MT-ADA chip as described in the following section.

Characterization of endogenous human rhIL21R-Fc fusion protein-specific reactivity in RA & SLE sera using the Maverick human MT-ADA PRI chip

The endogenous human rhIL21R-Fc protein-specific reactivity observed in the RA and SLE sera was further characterized by using a customized human MT-ADA isotyping chip. Use of a customized sensor system enabled investigation of the following questions: domain specificity of the anti-rhIL21R-Fc protein reactivity; determination of whether the reactivity was immunoglobulin in nature; isotype characterization of the reactivity.

Sensor chips were modified to confirm whether the detected reactivity was directed against the receptor domain versus the Fc domain of the rhIL21R-Fc protein. Channel 1 of the sensor contained clusters functionalized with rhIL21R-Fc protein, recombinant human rhIgG1-Fc, rhEphA1-Fc or rhMAdCAM-1-Fc, containing highly homologous or identical human Fc domain to that found on the rhIL21R-Fc protein. Channel 2 contained

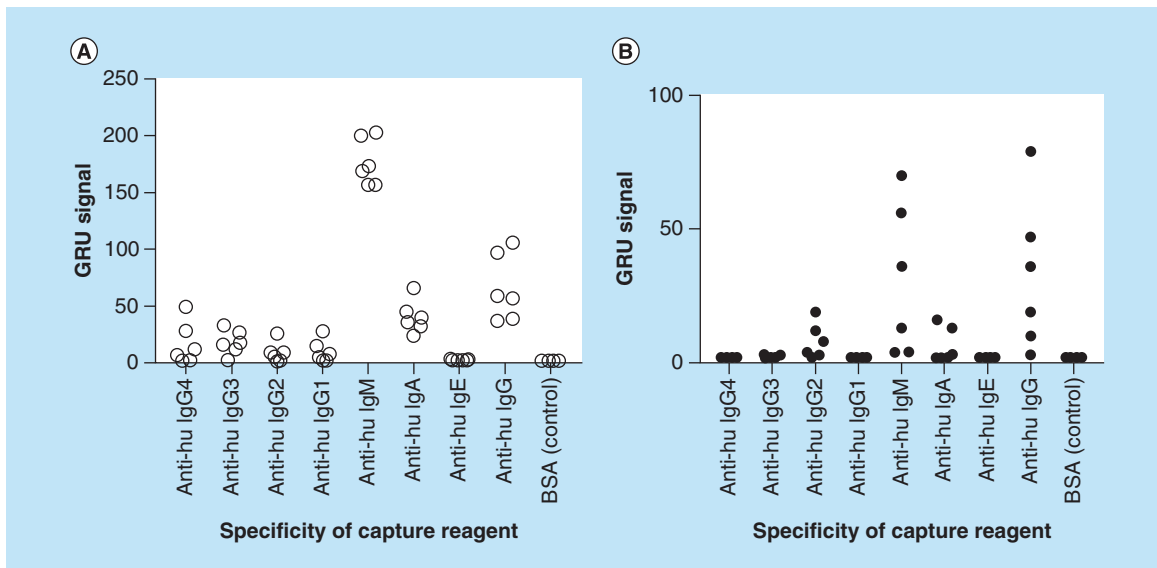


Figure 5. Isotype identification of anti-rhIL21R-Fc fusion protein reactivity. Isotype characterization of the rhIL21R-Fc fusion protein reactivity detected in the rheumatoid arthritis (A) and systemic lupus erythematosus (B) samples. Individual rheumatoid arthritis and systemic lupus erythematosus samples were tested using isotyping human Maverick biosensor chip. Six samples of each population were tested. BSA: Bovine serum albumin; GRU: Genalyte response unit.

the standard Maverick human MT-ADA isotyping multiplex arrangement including reagents specific for human immunoglobulin isotypes IgG, IgM, IgA, IgE as well as IgG subclasses (IgG₁, IgG₂, IgG₃, IgG₄).

Representative RA and SLE individual samples with a wide range of signals observed in the initial binding experiment were selected for further characterization using the custom biosensor chip in the Maverick MT-ADA protocol. Based on the level of observed background, a sample was deemed positive for the rhIL21R-Fc protein-specific reactivity if the generated GRU values were more than 4 units (based on a twofold increase over the background signal of two GRU observed for the bovine serum albumin [BSA] control). All selected RA and SLE samples scored positive when analyzed using sensors containing rhIL21R-Fc reagent. However, there was a distinct difference in the isotype patterns between the RA and SLE samples.

All analyzed RA samples scored positive, for example, showed binding to at least one sensor, when tested on the antihuman total IgG, IgG₄, IgG₃, IgG₂, IgG₁, IgM and IgA sensors (Figure 5A). IgM and total IgG isotype activity were particularly pronounced while IgE isotype was not detected. This result is not unexpected as RA serum samples are expected to contain rheumatoid factor components with antihuman Fc specificity of various isotypes including IgM, IgA and IgG [3,17]. All the tested RA samples scored positive using sensors containing rhIgG1-Fc reagent, indicating that the anti-rhIL21R-Fc protein reactivity is likely to be Fc domain specific (Figure 6A). RA samples tested using various Fc domain containing receptors scored positive against rhEphA1-Fc and the rhMAdCAM-1-Fc confirming this conclusion.

In contrast, analyzed SLE samples displayed a more variable pattern of isotype and domain specificity. SLE samples scored positive for the presence of IgG₂ (and correspondingly scored positive on the total IgG sensor), IgM and IgA isotypes (Figure 5B). It is not known why comparatively higher signals were observed on the total IgG sensor versus that for the IgG₂ subclass sensor with no other IgG subclass sensors scoring positive. It is possible that the observed difference was due to the specific binding affinity of the reagents used in the test. SLE reactivity was also distinct from that observed in the RA samples based on the domain specificity test. While all six RA samples showed specificity to the Fc domain-containing reagents, limited binding specificity against the rhIgG1-Fc and rhEphA1-Fc was observed in SLE samples (Figure 6B). More samples scored positive on the rhMAdCAM-1-Fc sensor indicative of a polyclonal nature of the reactivity.

Since the non-Fc portion of the IL21R-Fc protein was not available as a reagent, it is not known if the anti-rhIL21R-Fc reactivity detected in the RA or SLE samples was also directed against the non-Fc portion of the IL21R-Fc protein. It should be noted that the raw assay signal (GRU values) obtained in the test should not be

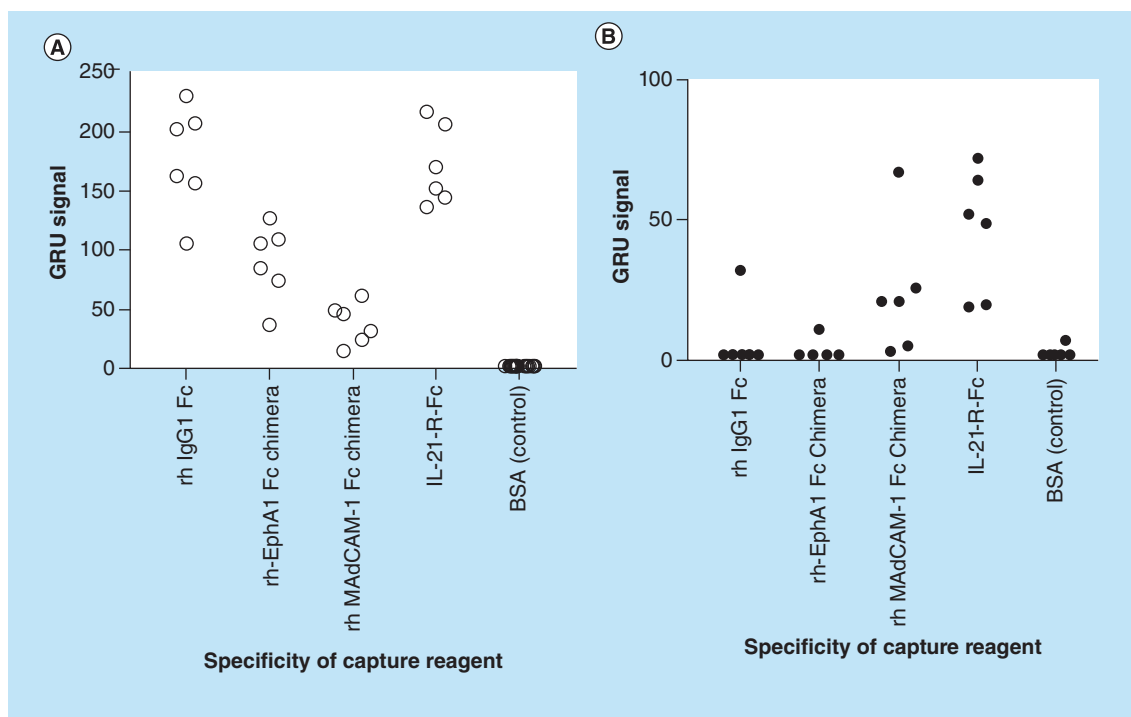


Figure 6. Domain specificity identification of anti-rhIL21R-Fc fusion protein reactivity. Domain specificity characterization of the rhIL21R-Fc fusion protein specific reactivity detected in the rheumatoid arthritis (A) and systemic lupus erythematosus (B) samples. Individual rheumatoid arthritis and systemic lupus erythematosus samples were tested using Maverick biosensor chip functionalized with rhIgG1-Fc, rhEphA1-Fc, rhMAdCAM-1-Fc, rhIL21R-Fc or BSA (control) reagents. Six samples of each population were tested. BSA: Bovine serum albumin; GRU: Genalyte response unit.

compared directly across the RA and SLE populations. A more appropriate comparison should be based on the number of positive and negative samples observed for each of the population types.

Discussion

Detection and characterization of antidrug-specific reactivity, including antidrug-specific antibodies, is an integral part of immunogenicity response evaluation for biotherapeutic compounds. Detection and characterization of drug-specific preexisting reactivity may be of significant value to facilitate assessment of drug immunogenicity potential as well as aid in the development of various bioanalytical methods. Domain and isotype specificity characterization may be important during evaluation of postdose immune response to a biotherapeutic. Access to a simple robust approach allowing detection and characterization of drug-specific reactivity in treatment naive and postdose samples is therefore important. In this report, we demonstrate the utility of a multiplexed immunoassay format using the Maverick Detection System to screen and characterize reactivity specific to two recombinant human proteins in normal and disease human matrices. While presented examples are based on application of the Maverick based platform, the main focus of the paper is to demonstrate the benefits of multiplexed immunoassay format during detecting and characterization of ADA like reactivity in unknown sample. Early detection and characterization of preexisting ADAs may greatly facilitate process of bioanalytical assay development. Two model protein systems were chosen for the system evaluation: the IgE and IL21R-Fc based. The IgE and anti-IgE interaction was intended to model a common concern of preexisting ADA interference, including anti-monoclonal antibody drug reactivity. In the second example, rheumatoid factor (RF) like reactivity binding to the Fc domain of the IL21R-Fc protein was detected and characterized.

In the tests designed to detect antihuman IgE reactivity, the mouse MT-ADA isotype Maverick PRI sensor chip correctly identified samples containing murine IgG1 subclass antihuman IgE antibody. Signal intensity generated in the PRI method generally correlated with the concentration of the murine antihuman IgE antibody spiked into.

In a separate case study, endogenous reactivity specific to the human rhIL21R-Fc fusion protein was detected in addition to the exogenously spiked specific ligand, human IL21. Use of the ACE procedure ensured specificity of the detected reactivity. Application of a customized MT-ADA assay sensor chip detected differences in the anti-rhIL21R-Fc-specific reactivity profiles in the tested RA and SLE samples. RA samples were shown to contain IgM, IgG (and IgG subclasses) and IgA isotype immunoglobulins specific to the rhIL21R-Fc protein, while limited IgM and IgG2 isotype reactivity was detected in the SLE patient samples. Use of a multiplexed immunoassay format, based on a customized MT-ADA assay sensor chip, allowed for simultaneous screening and characterization of domain specificity of the anti-rhIL21R-Fc reactivity. It was demonstrated that the reactivity detected in the RA samples was primarily specific to the Fc domain of the human rhIL21R-Fc fusion protein and is likely to be related to the presence of rheumatoid factors frequently observed in RA sera [17]. In contrast to the binding to all the human IgG1-Fc containing sensors by the RA samples, tests demonstrated that the majority of the six SLE samples did not bind to the rhIgG1-Fc and rhEPhA1-Fc sensors.

Conclusion

We have demonstrated that the primary benefit of a multiplex platform is the ability to simultaneously detect and characterize an unknown reactivity in a sample. The ability of a multiplex immunoassay format platform to identify immunoglobulin isotype and detect domain specificity of an antidrug reactivity in a multiplex protocol was shown to be an effective and efficient approach when screening and characterizing reactivity to a biotherapeutic candidate.

Future perspective

Characterization and evaluation of preexisting drug-binding antibodies has become a frequent necessity. The nature and specificity of preexisting antibodies is a part of this evaluation. As a result, we anticipate an increasing demand on sample volume and sample analysis turnaround. A multiplexed approach for this assessment is very attractive and efficient. We predict such an approach to be broadly applicable.

Summary points

- Screening and characterization of antidrug antibody like binding reactivity in normal human serum demonstrated that proposed platform approach can correctly identify the presence and nature of the antihuman IgE-binding component.
- Screening of binding reactivity against the human IL21R-Fc fusion protein via direct-binding Photonic Ring Immunoassay identified an unknown endogenous reactivity in the rheumatoid arthritis and systemic lupus erythematosus sera that was characterized for the domain specificity and immunoglobulin isotype by applying a customized multiplexing sensor system.
- The multiplexing nature of the platform applied for characterization allowed for a reduced overall analysis time. Based on examples presented herein, a multiplexed approach can productively and efficiently identify isotype and domain specificity of the drug-binding antibody.
- While the results here were produced using the Maverick Detection System, we believe that the conclusions are applicable to other existing immunoassay platforms with multiplexing capabilities.

Financial & competing interests disclosure

All the authors are or were former employees of Pfizer Inc. or Genalyte Inc. and may own respective stock or other form of equity. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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References

- 1 Mire-Sluis AR, Barrett YC, Devanarayan V *et al.* Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. *J. Immunol. Methods* 289(1–2), 1–16 (2004).
- 2 Kumar SC, DelCarpini JA, Qu Q, Kane M, Gorovits B. Mitigation of pre-existing antibodies to a biotherapeutic in non-clinical species when establishing anti-drug antibody assay cutpoint. *AAPS J.* 19(1), 313–319 (2017).
- 3 Tatarewicz S, Miller JM, Swanson SJ, Moxness MS. Rheumatoid factor interference in immunogenicity assays for human monoclonal antibody therapeutics. *J. Immunol. Methods* 357(1–2), 10–16 (2010).

- 4 Araujo J, Zocher M, Wallace K, Peng K, Fischer SK. Increased rheumatoid factor interference observed during immunogenicity assessment of an Fc-engineered therapeutic antibody. *J. Pharm. Biomed. Anal.* 55(5), 1041–1049 (2011).
- 5 Xue L, Fiscella M, Rajadhyaksha M *et al.* Pre-existing biotherapeutic-reactive antibodies: survey results within the American Association of Pharmaceutical Scientists. *AAPS J.* 15(3), 852–855 (2013).
- 6 Koren E, Smith HW, Shores E *et al.* Recommendations on risk-based strategies for detection and characterization of antibodies against biotechnology products. *J. Immunol. Methods* 333(1-2), 1–9 (2008).
- 7 Gorovits B, Wakshull E, Pillutla R, Xu Y, Manning MS, Goyal J. Recommendations for the characterization of immunogenicity response to multiple domain biotherapeutics. *J. Immunol. Methods* 408, 1–12 (2014).
- 8 Tatarewicz SM, Juan G, Swanson SJ, Moxness MS. Epitope characterization of pre-existing and developing antibodies to an aglycosylated monoclonal antibody therapeutic of G1m17,1 allotype. *J. Immunol. Methods* 382(1–2), 93–100 (2012).
- 9 Rispens T, de Vrieze H, de Groot E *et al.* Antibodies to constant domains of therapeutic monoclonal antibodies: anti-hinge antibodies in immunogenicity testing. *J. Immunol. Methods* 375(1-2), 93–99 (2012).
- 10 Mudumba S, de Alba S, Romero R *et al.* Photonic ring resonance is a versatile platform for performing multiplex immunoassays in real time. *J. Immunol. Methods* 448, 34–43 (2017).
- 11 Iqbal M, Gleeson MA, Spaugh B *et al.* Label-free biosensor arrays based on silicon ring resonators and high-speed optical scanning instrumentation. *IEEE J. Sel. Top. Quantum Electron.* 16(3), 654–661 (2010).
- 12 Fu Q, Zhu J, Van Eyk JE. Comparison of multiplex immunoassay platforms. *Clin. Chem.* 56(2), 314–318 (2010).
- 13 Mora J, Chunyk AG, Dysinger M *et al.* Next generation ligand binding assays – review of emerging technologies’ capabilities to enhance throughput and multiplexing. *AAPS J.* 16(6), 1175–1184 (2014).
- 14 Thermo Scientific, EZ-Link Sulfo NHS-LC biotinylation kit Manufacturer’s recommendations. www.thermofisher.com/order/catalog/product/21435
- 15 Thermo Scientific, Zeba Pak columns, Manufacturer’s recommendations. www.thermofisher.com/order/catalog/product/87766?SID=srch-srp-87766
- 16 Bourdage JS, Cook CA, Farrington DL, Chain JS, Konrad RJ. An affinity capture elution (ACE) assay for detection of anti-drug antibody to monoclonal antibody therapeutics in the presence of high levels of drug. *J. Immunol. Methods* 327(1-2), 10–17 (2007).
- 17 Conrad K, Roggenbuck D, Reinhold D, Dorner T. Profiling of rheumatoid arthritis associated autoantibodies. *Autoimmun. Rev.* 9(6), 431–435 (2010).