Quantification of MabSelect SuRe ligand in presence of excess amounts of IgG on Gyrolab

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Conclusions

- A prototype assay for MabSelect SuRe ligand in presence of excess amounts of IgG has been developed on Gyrolab
- Standards and samples are pretreated by acid dissociation for 15 min prior to analysis and the capture of MabSelect SuRe ligand is performed under acid conditions
- The assay for MabSelect SuRe operates at pH 3.5 at an IgG concentration of up to 0.6 g/L capable of detecting ~1 ppm (w/w) of MabSelect SuRe ligand
- Evaluation of assay performance including recovery on real process related samples as well as evaluation of different species of recombinant therapeutic IgG remain

Introduction

One very efficient step for large scale purification of recombinant therapeutic antibodies is affinity chromatography using immobilized protein A. Purification procedures based on usage of native protein A, either produced by staphylococci or recombinant E.coli have been available for decades. The binding of native protein A to IgG may involve two principally different mechanisms of interaction, Fc or Fab (V_HIII) (1, 2), forming complexes of different composition. Recently a new protein A ligand, MabSelect SuRe™, was introduced that is modified with respect to binding specificity as well as resistance to sanitization of chromatographic resins at alkaline pH. MabSelect SuRe binds effectively to the Fc portion of IgG1, IgG2 and IgG4 but the Fab-binding property is essentially abolished leading to a more homogenous chromatographic behavior of different monoclonal IgGs (3).

Protein A ligands may leach from chromatographic resins, particularly during elution at acid pH, and contaminate the IgG preparation. Quantification of protein A ligand in presence of huge amounts of IgG pose significant problems due to complex formation and reduced antigen accessibility at neutral pH. Several approaches have been tried to overcome these problems, either by performing the protein A assays under acid conditions (4) or by heat denaturation of IgG by boiling samples in presence of various additives (5).

There are several problems that must be addressed when designing an assay for quantification of protein A ligands in presence of IgG.

- Which antibodies are optimally reactive with the selected protein A ligand to generate a sufficiently sensitive assay?
- At which pH is protein A ligand dissociated from IgG?
- In which pH range is the assay operational?
- How does presence of IgG affect performance of assay?

Here we report a prototype assay protocol for quantification MabSelect SuRe ligand in presence of IgG, a ligand which is commonly used for development of new purification processes of recombinant therapeutic IgG.

Gyrolab Technology

Gyrolab is a new, bioanalytical platform which is designed for miniaturized immunoassay and performed in disposable CDs containing microstructures in which samples are analyzed in parallel. Each microstructure contains a 15 nl column of streptavidin coated particles on which reactions take place. The principles for miniaturizing and integrating assays onto a Bioaffy CD are illustrated in Figure 1.

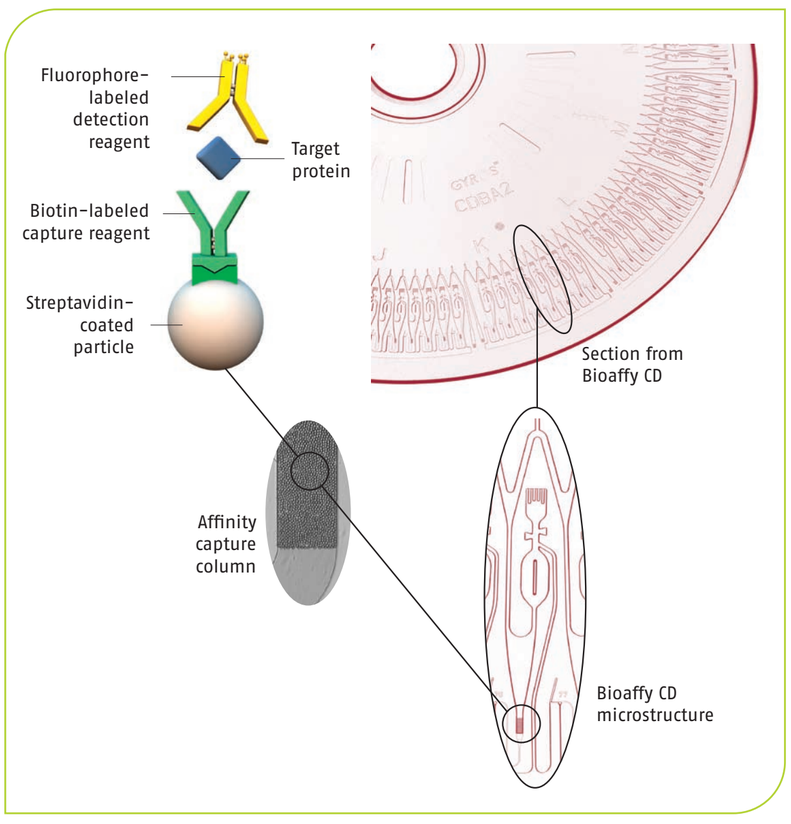


Fig. 1. Sandwich-based immunoassays miniaturized and integrated into a CD microlaboratory.

CDs are processed in Gyrolab. Samples and reagents are transferred sequentially to specific inlets on the CD by a robotic arm. Liquid enters the microstructures by capillary action and the required volumes are defined within each microstructure. Samples and reagents are moved through the microstructure by spinning the CD at pre-programmed intervals and speeds to create the desired flow rates. Detection begins as soon as the reactions are completed using a laser-induced fluorescence detector integrated in the workstation. Each Bioaffy CD takes approximately one hour to process and generates 96 data points. Assays are created by sequential addition of reactants and processed in flow-through mode. All interactions occur under flow conditions reducing non-specific interactions favoring high signal:background ratios and wide assay working ranges. The open nature of the platform combined with rapid processing makes it attractive for various analytical situations.

Experimental

Reagents

An affinity purified chicken antibody directed against native protein A (code no A135-Prot-A-AF) was purchased from Norwegian Antibodies, Aas, Norway. Chicken antibody was labeled with biotin (Cat no 21335, Pierce, Rockford, IL) and Alexa Fluor 647 (Cat no A-20186, Molecular Probes, Eugene, OR) respectively, according to manufacturers recommendations. Labeled antibodies were used in concentrations 100 µg/ml (capture reagent) and 12.5 nM (detecting reagent).

A monoclonal antibody was obtained in biotinylated form (Cat no B 3150, Sigma-Aldrich, Saint Louis, MI) and was used in combination with Alexa labeled chicken antibody.

MabSelect SuRe ligand was obtained from GE Healthcare, Uppsala, Sweden (Code no 28-4018-60). Native protein A produced in *S. aureus* was obtained from GE Healthcare (Code no 17-0872-05).

Experiments were performed in Bioaffy 200 and Bioaffy 1000 CDs.

Sample preparation

Standards were prepared in two steps by mixing constant amounts of monoclonal IgG at neutral pH with varying concentrations of protein A ligand ranging from 120 to 0.164 ng/ml at a final IgG concentration of 2.4 g/L. Prior to analysis, samples were diluted 1/4 in 0.3 M citrate buffers at different pH ranging from 3.2–3.6 to generate standard curves at the selected pH containing constant amounts of IgG (0.6 g/L) and varying amounts of protein A ligand (0.041–30 ng/ml).

IgG

A commercially available recombinant human monoclonal IgG1κ antibody (Product no AB002) was purchased from Polymun Scientific Immunbiologische Forschung GmbH (Vienna, Austria).

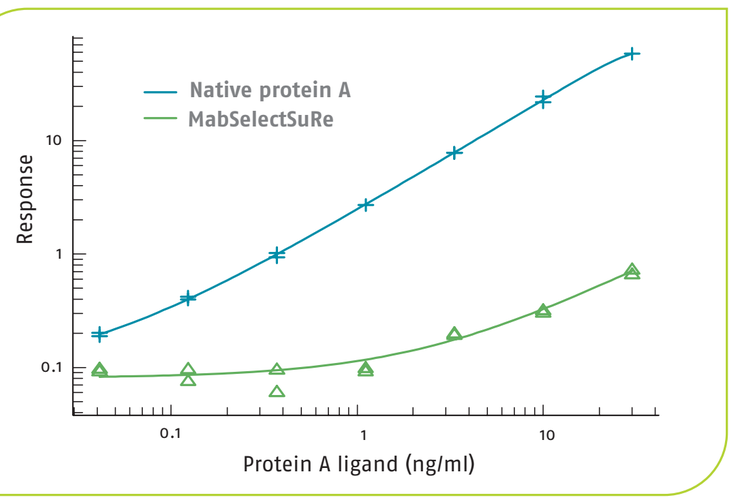
Results

Selection of antibodies and assay conditions

Optimization of the assay for MabSelect SuRe ligand followed the experimental order below:

- Selection of antibodies based on assay performance in absence of IgG
- Investigation of assay performance in relation to assay pH
- Investigation of assay performance at selected pH and at an IgG concentration of 0.1–1 g/L

Since MabSelect SuRe ligand has been genetically modified compared to native protein A, it was not obvious that reagents prepared against native protein A would react equally well with MabSelect SuRe ligand. Two different combinations of antibodies were used to find the best reagent set up for assaying native protein A and MabSelect SuRe ligands. Figure 2 and 3.



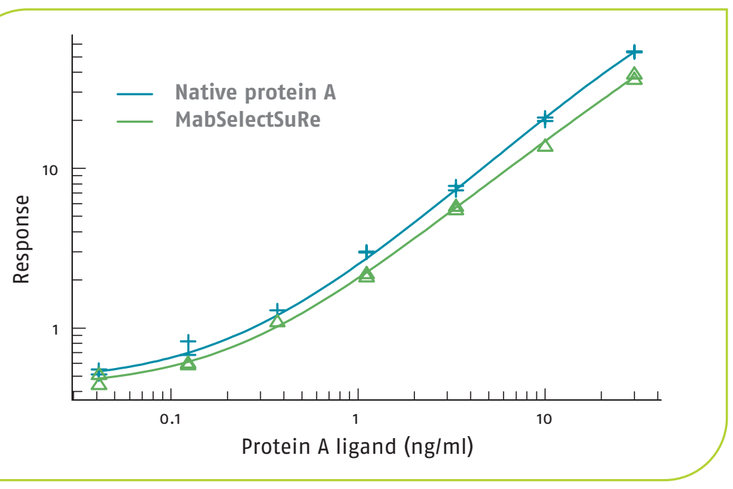


Fig. 2 and 3. Different combinations of antibodies were tested in putative assays for native protein A (Mouse/Chicken) and MabSelect SuRe (Chicken/Chicken) at pH 3.4 in absence of IgG.

The efficiency of capturing MabSelect SuRe ligand in different acid environments using different pH in 0.3 M citrate buffers ranging from and 3.2–3.6 (Fig 4.) The assay for MabSelect SuRe ligand is significantly affected at pH below 3.5. Thus it was concluded to perform the assay for MabSelect SuRe at pH 3.5, a pH lower than what is normally used for elution of IgG from chromatographic resins utilizing MabSelect SuRe (3).

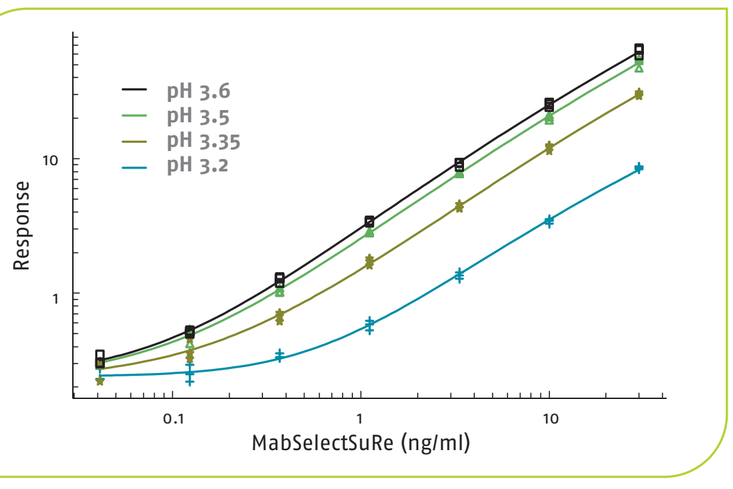


Fig. 4. Acid susceptibility for MabSelect SuRe ligand using Chicken/Chicken antibody based assay.

Gyrolab method

During the initial phase of assay development the importance of maintaining the selected pH throughout washing steps of the capture column subsequent to protein A ligand capture became evident. Thus several wash steps using buffers at the selected acid pH was added in the method to prevent rebinding of trace amounts of IgG remaining in the upper parts of the microstructure before neutralizing pH. The final method is comprised of 16 separate additions of liquid in which a block of acid washes is introduced prior to and after the capture step of MabSelect SuRe ligand. The final design of the method is described in Fig 5.

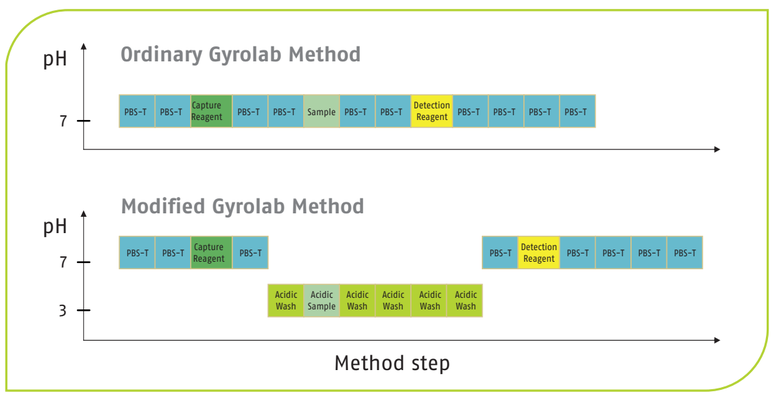


Fig. 5. New Gyrolab method for MabSelect SuRe ligand assay.

Assay performance at acid pH and in presence of IgG

Optimally, under the conditions selected, the assay for MabSelect SuRe ligand should be unaffected by presence of IgG in samples. In experiments aimed at evaluating the effect of monoclonal IgG on the performance of MabSelect SuRe ligand assay in Bioaffy 1000, a standard curve containing constant amounts of human IgG at 0.6 g/L was analyzed. For comparison MabSelect SuRe ligand was also analyzed in absence of IgG (Fig.6). Despite the acid conditions used, the assay is influenced by presence of IgG reducing assay sensitivity by approximately one order of magnitude. The assay capability of detecting MabSelect SuRe ligand in presence of IgG is approximately 1 ppm (w/w).

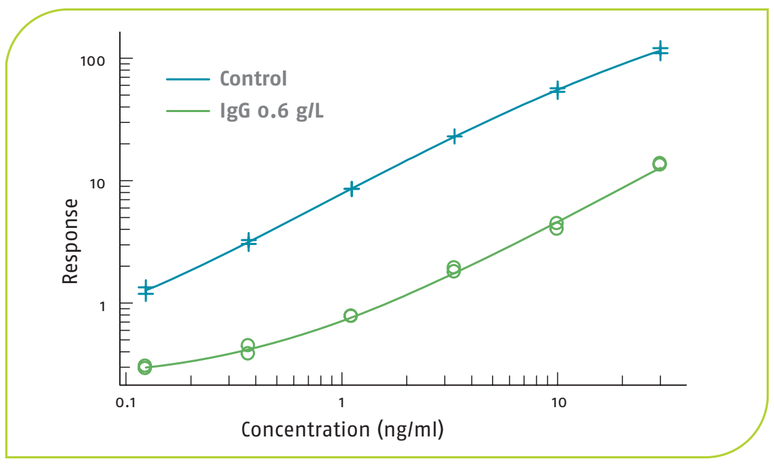


Fig. 6. Assay performance for MabSelect SuRe ligand in presence of 0.6 g/L of recombinant human monoclonal IgG at pH 3.5. For reference the MabSelect SuRe ligand is analyzed in absence of IgG.

Summary of assay conditions

A principal illustration of the assay conditions selected in relation to immuno-reagent performance and accessibility of MabSelect SuRe ligand in Fig 7.

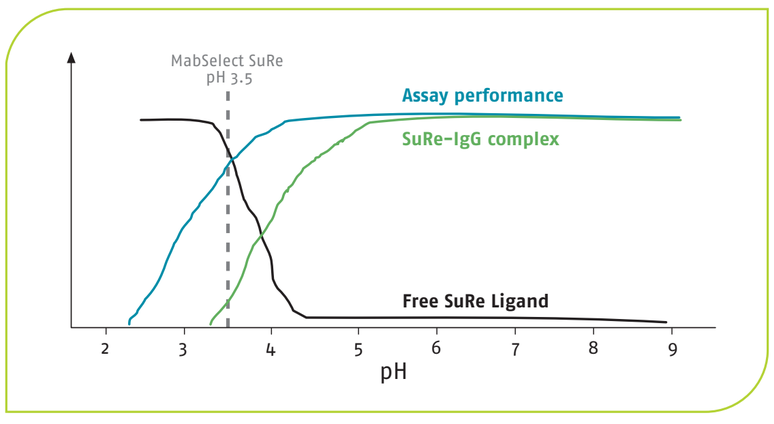


Fig. 7. Illustration of factors impacting performance of MabSelect SuRe assay

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