1. Introduction

Sartorius has developed a platform ELISA to measure Fab arm exchange (FAE) of IgG4 antibodies. IgG4 molecules are unique among the IgGs in that they can undergo FAE to form bispecific antibodies. Half-molecules of one IgG4 recombine with half-molecules of other IgG4 molecules to form antibodies with two specificities. This occurs in vivo and can also occur in vitro by adding mild reducing agents. There are two sites in the IgG4 molecules that are involved in determining the FAE: S228 in the core hinge and R409 in the CH3 domain. The ability of therapeutic IgG4 antibodies to potentially exchange with endogenous IgG4 antibodies in patients could lead to off-target effects. Some IgG4 antibodies, such as natalizumab, contain a mutation in the hinge region, S228P, that prevents this exchange from occurring. In contrast, natalizumab does not contain this mutation.

2. Experimental Setup

This assay utilizes a sandwich-based ELISA. By coating the plate with the target of one monoclonal antibody and detecting the other target is achieved by using an anti-idiotypic antibody, which will either not bind to the target on the ELISA plate, or they will not be detected by the anti-idiotypic antibody.

3. Development using MODDE®

Using MODDE® Pro software, a full factorial design of experiment approach was used to begin development of the ELISA. There were two factors assessed: three concentrations of anti-idiotypic antibody and three concentrations of HRP antibody.

4. Results – MODDE®

Output from MODDE® demonstrated the parameters required to achieve the optimum upper asymptote result. As shown in Figure 3, the lower concentrations of HRP result in the higher upper asymptote values. Figure 4 demonstrates the graphed data from Table 1, which was used to populate the information for MODDE® to run the analysis.

5. Results – Wild-Type IgG4 Antibodies

Two wild-type IgG4 antibodies without the S228P mutation were incubated with the reducing agent, Glutathione (GSH). They were subsequently tested in the ELISA shown in Figure 2. There was detection of bispecific antibodies in both the 5 mM and 0.5 mM GSH-treated samples, indicating Fab arm exchange had occurred.

6. Results – Mutated IgG4 Antibodies

One wild-type IgG4 antibody and one IgG4 with the S228P mutation were incubated with the reducing agent, GSH. They were subsequently tested in the ELISA shown in Figure 2. There was detection of bispecific antibodies in both the 5 mM and 0.5 mM GSH-treated samples, indicating Fab arm exchange had occurred.

7. Conclusion

The data presented here demonstrates that Fab arm exchange in the ELISA used can be detected by a sandwich ELISA method. After incubation with reducing agents, the IgG4 antibodies create bispecific antibodies that can be exploited to detect both Fab regions. The plate is coated with one target, and detection of the other target is achieved by using an anti-idiotypic antibody, which will not bind to the target on the ELISA plate, or they will not be detected by the anti-idiotypic antibody. This is particularly useful for detecting Fab arm exchange in IgG4 antibodies with the S228P mutation.