Rapid development of a GMP-compliant assay for the determination of antibody concentration

- Easy, rapid validation of assay performance
- Assays compliant with current ICH guidelines

Introduction

Manufacturing regulations require that the concentration of proteins produced by recombinant or hybridoma technology is monitored throughout the production process using validated assays. Here, we describe the process leading to the establishment of a validated assay for determining the concentration of a monoclonal, fully human anti-EpCAM IgG1 antibody (Naundorf *et al.,* 2002) intended for the treatment of prostate cancer, from harvest samples, process controls and final product. The conditions were optimized within three months to deliver an accurate and reliable assay.

Assay validation: preliminary conditions

Although no definitive guidelines on the validation of binding data are available today, the FDA documents, *Guidance for Industry Bioanalytical Method Validation and Guidance for Industry: Analytical Procedures and Methods Validation Chemistry, Manufacturing, and Controls Documentation* provide generally accepted guidelines for binding assay design. Until consensus is established, individual QA departments are required to comply with the current ICH guidelines for assay validation.

Once the basic design of the assay is decided, the precise conditions under which it will be run must be determined empirically. Here, an assay was constructed according to the FDA guidelines above, in which a humanized monoclonal antibody is captured on Sensor Chip CM5 after initial immobilization of protein A serving as the IgG-specific binding molecule. Immobilization, surface regeneration, and sensor chip storage were all investigated and the optimal conditions for the final assay were determined from these data. All experiments were performed using certified Sensor Chip CM5 and a Biacore™ C system.

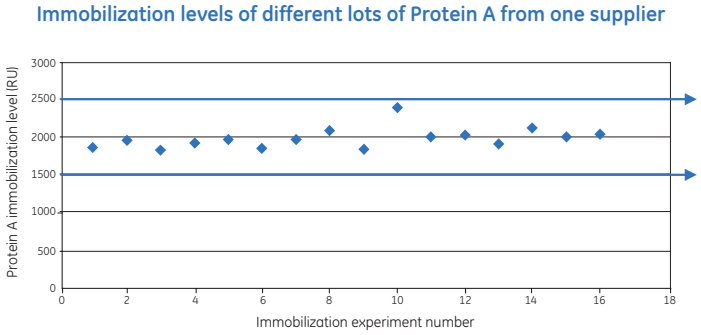

Figure 1. Immobilization levels of 16 lots of protein A from a single supplier on certified Sensor Chip CM5. The mean immobilization level using 10 µg/ml protein A at a flow rate of 5 µl/minute for 7 minutes was 1970 ± 137 RU (CV 7.0%).

Optimization of protein A immobilization conditions

Protein A was immobilized on certified Sensor Chip CM5 in order to measure the consistency of immobilization levels between different batches. Sixteen lots of protein A prepared at a concentration of 10 µg/ml and injected over the sensor chip at a flow rate of 5 µl/minute for 7 minutes gave immobilization levels of between 1500 and 2500 RU (Figure 1). There were no significant differences in the immobilization levels of different lots of protein A under these conditions. It is recommended that routines are established to achieve a similar level of protein A immobilization before further assay optimization.

Acknowledgement

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A concentration range of monoclonal antibody MT201 was then passed over each flow cell and binding levels were measured. Optimal binding of MT201 was achieved using protein A immobilized under the conditions described above. Further binding of MT201 was not seen by increasing immobilized protein A beyond this level (Figure 2).

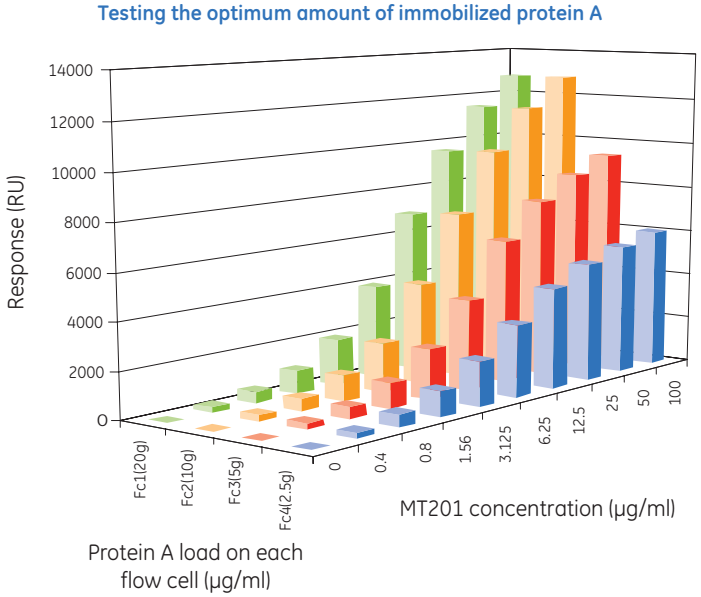


Figure 2. Optimization of protein A immobilization conditions. Maximal binding of MT201 to protein A was achieved using 10 µg/ml protein A at a flow rate of 5 µl/minute for 7 minutes.

Optimization of regeneration buffer

More than 20 buffers were screened in various combinations. Rapid regeneration was achieved using a single 30 second injection with 0.5 M NaSCN containing 10 mM NaOH (Figure 3).

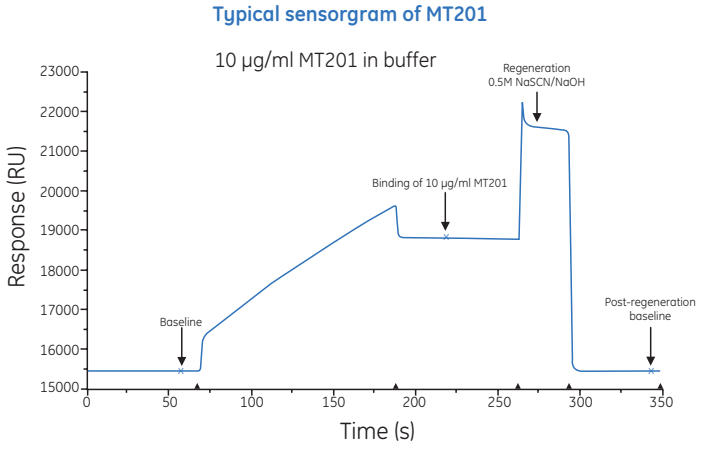


Figure 3. Optimization of regeneration buffer. 0.5 M NaSCN in NaOH gave good regeneration (compare post-regeneration baseline with original baseline) with a C.V. of less than 1.5% over several dozen runs. The decision to finally select 0.5 M NaSCN in NaOH was based on speed and a desire to select the least aggressive chemical regime that still provides acceptable regeneration.

Optimization of chip storage conditions

After binding of MT201 to the capturing molecule, the stability of the surface was compared between chips stored in air at room temperature and those stored in HBS-EP buffer containing sodium azide (Biacore AB, a GE Healthcare company, Sweden). The surface activity of exposed sensor chips was reduced by up to 50% after one week and by up to 70% after 2 weeks. In contrast, chips stored in HBS-EP buffer remained fully active even after 2 months (Figure 4).

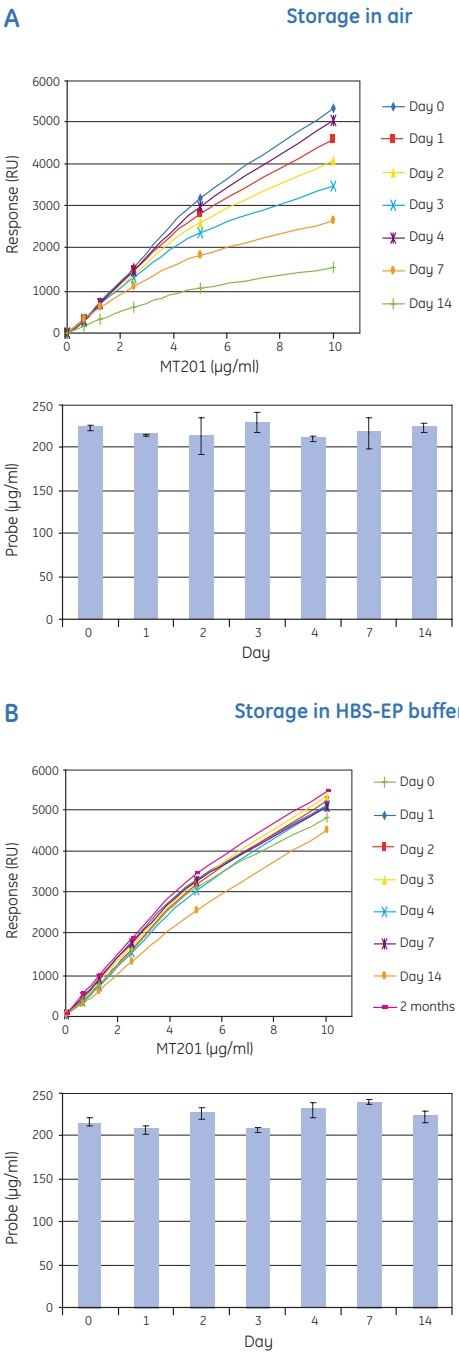


Figure 4. Optimization of chip storage conditions. The effect of surface activity of certified Sensor Chip CM5 stored (A) in air or (B) in HBS-EP buffer.

Assay validation: experimental design

Robustness

Influence of surfactant: The presence of surfactant at concentrations as low as 0.001% in sample buffer had a significant effect on the signal. However, surfactant concentrations above 0.1%, the influence of surfactant on the signal leveled out, with negligible additional effects on R_{max} across a range of MT201 concentrations up to 10 µg/ml (Figure 5).

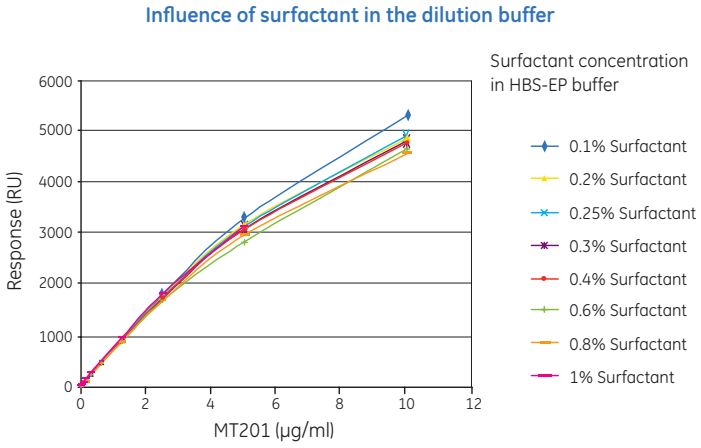


Figure 5. The influence of surfactant in dilution buffer. The assay is robust at surfactant concentrations over 0.1% in the dilution buffer.

Influence of protein A: In the purification process of MT201, the antibody is eluted from a protein A column. Minute quantities of protein A may contaminate the sample, with up to 50 ng/ml protein A expected to leach from the column. It was therefore important to know if trace amounts of contaminating, soluble protein A could interfere with the assay by binding MT201 in solution and inhibit the antibody from binding to capturing molecule protein A on the sensor chip. To test this, 0.016-25 ng/ml protein A solutions were spiked with 2.5 ng/ml MT201 and analyzed. Inhibition of the signal was seen only at protein A concentrations above 0.5 ng/ml after 1:500 column eluate dilution, well above concentrations expected after elution of antibody from the column.

Influence of buffer composition: Three alternate elution buffers were tested to measure their effect on concentration measurements. 1 mg/ml MT201 was added to the elution buffers and then diluted from these stocks to concentrations of 5, 2.5 and 1.25 µg/ml in six alternate sample buffers. The responses were compared with similar concentrations of MT201 added directly to the same six sample buffers. Bovine serum albumin (BSA) in the sample buffer competitively bound to protein A, greatly effecting the response at low concentrations of MT201 (Figure 6), suggesting that in this assay BSA should be omitted from the sample buffer as stabilizer.

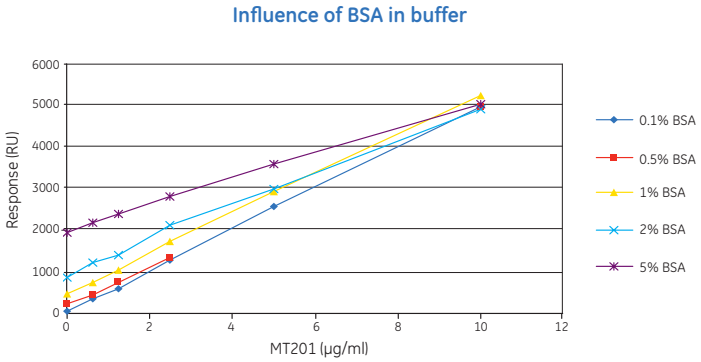
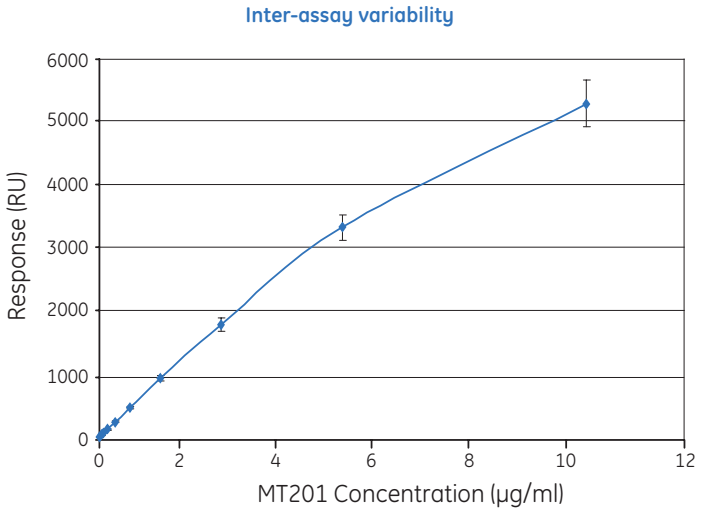


Figure 6. The influence of bovine serum albumin in sample buffer. These tests showed that BSA in the sample buffer competitively binds protein A, possibly masking signal generated by low concentrations of MT201 binding to protein A.

Inter-assay variability

A total of 26 assays were run on different days using four different sensor chips. Inter-assay variability was below 10% at the highest concentration of MT201 tested (Figure 7). All experiments were performed within seven days.



MT201 concentration (µg/ml)	R_{max} (RU)	SE (RU)	CV (%)
10	5225	357.7	6.8
5	3280.4	199.8	6.1
2.5	1770	96.2	5.4
1.25	924.9	33.3	3.6
0.625	467.4	17.8	3.8
0.313	233.9	10.5	4.5
0.156	120.3	6.6	5.4
0.078	63.3	3.9	6.2
0.039	33.6	2.7	8.1
0	2.1	0.9	NR

Figure 7. Inter-assay variability. In these validation tests, 1 chip (2 flow cells) and 3 chips (one flow cell each) were used. In total, 26 standard curves were generated over seven days. Dilution buffer was HBS-EP + 0.25% surfactant. NR: not reported.

Table 1. Intra-assay variability. Dilution buffer was HBS-EP containing 0.25% surfactant. NA: not applicable. NR: not reported.

5 µg/ml MT201			1.25 µg/ml MT201			0.08 µg/ml MT201		
Number of runs	R_{max}	CV (%)	Number of runs	R_{max}	CV (%)	Number of runs	R_{max}	CV (%)
6	3517	2.7	6	932	2.6	6	64	6.9
3	3512	2.4	3	962	1.1	3	64	1.3
6	3408	4.3	6	951	9.6	6	67	18.8
1	3193	NA	1	918	NA	1	NR	NA
2	3020	0.3	2	872	0.5	2	57	1.0
3	3216	2.1	3	950	1.1	3	67	4.0
-	-	-	3	891	0.8	3	62	1.4

Intra-assay variability

Different lots of Sensor Chip CM5 were tested over repeated measurements of 3 different concentrations of MT201 added to sample buffer containing 0.25% surfactant. Variability was below 5% in samples spiked with 5 µg/ml MT201, below 10% in samples spiked with 1.25 µg/ml MT201 and below 20% in those spiked with 0.08 µg/ml MT201 (Table 1).

Sensitivity (LOD/LOQ)

Close to the limit of quantitation, intra-assay variability naturally increases. From a linearity plot (samples run from 0.04 to 5 µg/ml MT201), the limit of quantitation (LOQ) is determined to be 80 ng/ml with CV below 20%. Over this concentration range, R^2 was consistently above 0.99 in six concentration runs using different lots of sensor chip.

Sample storage stability

Long term stability

Reference standards in six different buffers were frozen at -80°C and tested after different storage periods. Samples were stable for up to 6 weeks of freezing both in sample buffer containing 1% BSA and HBS-EP with 0.25% surfactant.

Stress testing of reference material

Comparisons were made between materials stored at -80°C, at 37°C for extended periods and samples subjected to up to 5 freeze/thaw cycles. None of these treatments affected the performance of the assay.

Short term stability

Comparisons were made between freshly diluted samples and samples diluted and stored at room temperature for 24 hours. Measurements were compared between HBS-EP buffer with 0.001% surfactant or 0.25%. Standards were tested using harvest, as well as protein A eluates, SP eluates, Q eluates and final bulk. At no point in the process was the performance of the assay significantly affected by short term storage of the sample at room temperature.

ELISA comparison

In a single direct comparison in which identical samples were measured using Biacore C and ELISA, Biacore C gave both lower standard deviations and variances in the data (Figure 8).

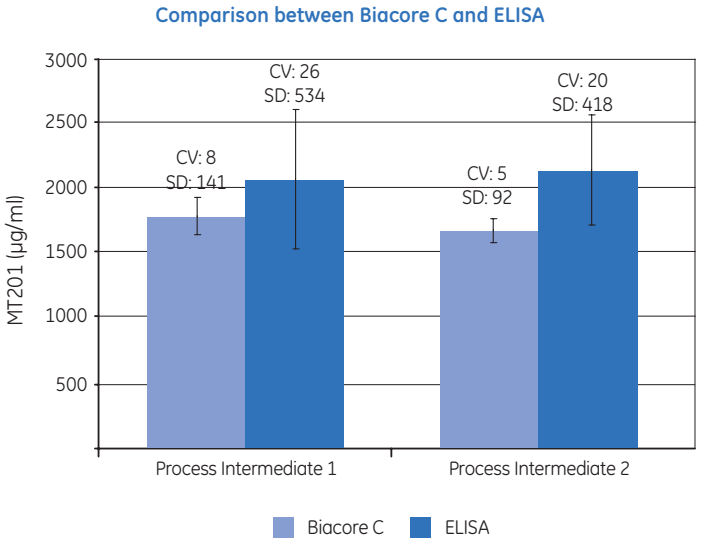


Figure 8. Comparison between Biacore C and ELISA. The results are from a direct side by side comparison at the same time.

Conclusions

Biacore C was selected as the instrument of choice for monitoring monoclonal antibody concentrations across the production process. Here, the quality of principal assay parameters such as specificity, accuracy, precision, robustness and range are shown to be easily and rapidly validated. Biacore C may be quickly qualified for use in concentration measurement applications in GMP-regulated laboratories.

References

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