THE DEVELOPMENT OF AN ELISA ASSAY FOR THE DETERMINATION OF CETUXIMAB IN HUMAN SERUM

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Introduction

Cetuximab (Erbitux®) is a chimeric monoclonal antibody of the immunoglobulin G1 (IgG1) class that is directed against the human epidermal growth factor receptor (EGFR). It is used in combination with chemotherapy for treatment of metastatic colorectal cancer and head and neck cancer.

The objective of this assay was to develop a cost-effective and accurate method to allow for a rapid validation of client-specific cetuximab biosimilar compounds. Historically, ELISAs for cetuximab have used EGFR as the capture molecule. EGFR is expensive and can be cost-prohibitive with studies involving a large number of samples. This method was developed using an anti-cetuximab antibody, significantly reducing the per-plate reagent cost.

Method

Diluted serum samples were added to a microplate previously coated with an anti-cetuximab capture antibody. The wells were washed to remove any unbound sample material and an enzyme-labeled antibody added. Unbound labeled antibody was removed and a chromogenic substrate added. The development of the colored reaction product was directly proportional to the amount of cetuximab present in the sample. The microplate was then analyzed using a colorimetric plate reader.

Results

A 4-parameter logistic auto-estimate regression weighted $1/Y^2$ was used for the analytical range 0.300–10.0 µg/mL of cetuximab in human serum. Cetuximab calibration standard concentrations were back-calculated from the regression equation and the inter-batch percent CV was less than 3.8.

Precision and accuracy of quality control samples were assessed on samples between 0.300 and 10.0 µg/mL. The inter-batch precision (percent CV) was less than 10.0 and the inter-batch accuracy (percent Bias) was between -7.3 and +2.3.

Table 1. Cetuximab Inter-Batch Precision and Accuracy

Cetuximab	LLOQ QC 0.300 µg/mL	Low QC 0.900 µg/mL	Mid QC 1.70 µg/mL	High QC 1.70 µg/mL	ULOQ QC 10.0 µg/mL
Inter-batch Mean	0.278	0.921	1.71	7.30	9.62
Inter-batch SD	0.0275	0.0364	0.101	0.263	0.483
Inter-batch % CV	9.9	3.9	5.9	3.6	5.0
Inter-batch % Bias	-7.3	2.3	0.6	-2.7	-3.8
n	9	9	9	9	9

The absence of a hook effect (an artifact causing samples with concentrations greater than the ULOQ to back-calculate within the analytical curve range) was demonstrated for cetuximab by assaying a sample with a concentration higher than the ULOQ. A sample with a concentration above the ULOQ was analyzed and quantitated above the acceptable response, indicating there is no hook effect associated with this method.

Hemolyzed sample integrity was performed at the mid-QC concentration in serum fortified with 5 percent whole blood. The percent bias using three different lots of hemolyzed matrix was less than -17.6, indicating that sample hemolysis does not have a significant impact on quantitation.

Matrix effect was evaluated in ten individual lots of human serum. Ten of ten lots of matrix fortified at the low and high QC concentrations, quantitated within +/- 20 percent of the respective theoretical concentrations.

Table 2. Matrix Effect for Cetuximab in Human Serum

	Low QC		High QC	
Lot#	0.900 µg/mL	% Dev.	7.50 µg/mL	% Dev.
1	0.862	-4.2	7.86	+4.8
2	0.861	-4.3	7.85	+4.7
3	0.875	-2.8	7.66	+2.1
4	0.911	+1.2	7.63	+1.7
5	0.887	-1.4	7.70	+2.7
6	0.882	-2.0	7.75	+3.3
7	0.807	-10.3	7.20	-4.0
8	0.829	-7.9	7.11	-5.2
9	0.836	-7.1	7.14	-4.8
10	0.877	-2.6	7.30	-2.7
Mean	0.863		7.52	
% CV	3.6		4.0	
% Bias	-4.1		0.3	
n	10		10	

Dilution integrity was evaluated and demonstrated samples could be diluted up to 10-fold to dilute them into the quantifiable range.

Combined short-term and freeze-thaw stability was established at the mid QC concentration for six freeze (-80°C) and thaw (ambient temperature) cycles and a total of 24 hours at ambient temperature under white light, with a percent bias of -6.5.

Table 3. Short-term and Freeze-thaw Stability

	Mid QC 1.70 ug/mL
	1.57
	1.60
	1.61
Mean	1.59
% CV	1.3
% Bias	-6.5
n	3

Conclusion

A method for the analysis of cetuximab in human serum has been developed, and will allow for a rapid, cost-effective, accurate, and reproducible assay of cetuximab. This method is available for use in the comparative analysis of cetuximab and a biosimilar compound, and provides a much more cost-effective method compared to previously published methods.