W4282

Development and Qualification of a Novel Integrated - ELISA Assay for the Measurement of Infliximab (IFX) and Antibodies to Infliximab (ATI) Assays to Support Post-Marketing Surveillance Studies

R. Islam[1] and P. Banks[2]
[1]Celerion, Lincoln, NE USA and [2]Somru BioScience, Charlottetown, PE Canada

Background and Purpose

Infliximab (IFX) has revolutionized therapy in patients with chronic immune diseases mediated by pro-inflammatory cytokine tumor necrosis factor-alpha (TNF)-α, such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, and chronic severe plaque psoriasis. However, a significant number of patients have been reported to develop resistance to the therapy over time. The formation of anti-drug antibodies, which accelerates clearance of the drug, is believed to be the primary factor contributing to this observation. As for the consequences, a significant number of scientific publications demonstrated increased risk of loss of clinical response, disease relapse, impaired quality of life, and increased cost of care. Thus, there is an increased necessity to accurately monitor serum Infliximab (IFX) as well as to determine whether the presence of antibodies to Infliximab (ATI) is clinically relevant and directly correlated to the decreased drug efficacy.

In addition, both the Food and Drug Administration and European Medicines Agency require that manufacturers of biosimilars must have adequate post-marketing surveillance mechanisms in place to detect possible differences between the reference and biosimilar products. This requires monitoring ATI to prevent adverse reactions to the marketed biosimilar. It has also been proposed that IFX concentration should be monitored alongside the ATI as lower levels of circulating IFX is indicative of ATI formation. Thus, the knowledge of both IFX and ATI levels, provide a better insight into drug efficacy and safety.

Here we report a novel integrated-ELISA assay capable of measuring IFX and ATI levels simultaneously, in a cost-effective, sensitive and specific manner with minimal interference from diseased matrix components.

Methods

The IFX method utilizes antibodies against Infliximab that are generated in a chicken model. Chicken antibodies have several advantages over traditional mammalian antibodies (Table 1). One major advantage is that chicken antibodies do not cross-react with rheumatoid factors, which is expected to be present at a high concentration in the patient population.

Briefly, the method requires coating of the microtiter plates with streptavidin for both IFX and ATI detection. Samples are pre-treated with low pH buffer to minimize the impact of IFX on ATI measurement and vice versa. For the IFX method, biotinylated TNF-alpha is incubated with samples and the TNF-alpha-IFX complex is detected using hapten-conjugated chicken anti-IFX antibody. For the ATI assay, biotinylated-IFX is incubated with samples. The complex IFX-ATI is then detected using a cocktail of hapten-conjugated IFX and anti-human IgG4 antibody. This allows for the detection of IgG4-ATI as it is reported that a significant portion of ATI response is of IgG4 subclass. The traditional bridging ELISA is not capable of detecting IgG4 due to the monovalent nature of IgG4.

Both the IFX and ATI assays are designed to be performed in parallel using one microtiter plate. The following parameters were evaluated during the qualification of the IFX method: precision, accuracy, selectivity/ matrix effects, sensitivity, dilutional linearity, and interference from circulating TNF-alpha. For the ATI method the following parameters were evaluated during qualification: precision, cutpoint, selectivity, sensitivity, hook effect and drug tolerance.

Table 1. Advantages of Chicken IgY over Mammalian IgG.

	Chicken IgY	Mammalian IgG
Source	Egg Yolk	Plasma
Avidity	High	Moderate
Quantity	Large	Limited
Cross-Reactivity to Rheumatoid Factors, Complements and HAMA	None	High
Isolation	Rapid, Cost-Effective	Time-Consuming, Expensive
Productivity	High	Low
3R (Refinement, Reduction and Replacement)	Ideal	Not Ideal

Results

Table 2. Inter-Batch Precision of Calibration Curve.

Inter-Batch	Calibrators-Concentration of IFX (ng/mL)							
	5000	4000	2500	1250	625	312	156	78
Mean	4798	3903	2537	1212	640	308	166	66
SD	509	164	185	45	24	14	22	13
%CV	11	4	7	4	4	5	13	20
%Bias	-4	-2	1	-3	2	-1	6	-16
n	6	6	6	6	6	6	6	6

Table 3. Inter-Batch Accuracy and Precision of Validation Samples.

Inter-Batch	Concentration of IFX (ng/mL)				
	5000	3750	1500	450	156
Mean	4679	3945	1584	440	153
SD	190	289	86	24	27
%CV	4	7	5	5	18
%Bias	-6	5	6	-2	-2
n	7	7	7	7	6

Table 4. Summary IFX Validation Data.

Assay Parameter	Results
Analyte	Infliximab
Limit of Quantitation (ng/mL)	156 ng/mL
Calibrator Concentrations (ng/mL)	5000, 4000, 2500, 1250, 625, 312,156, 78 (anchor point)
Regression Type	5PL, $1/Y^{\wedge 2}$
QC Concentrations (ng/mL)	156 (LLOQ), 450 (Low), 1500 (Mid), 3750 (High), 5000 (ULOQ)
Intra-Batch Precision Range (%CV) - QCs	2% to11%
Intra-Batch Accuracy Range (%Bias) - QCs	-3% to 7%
Inter-Batch Precision Range (%CV) - QCs	4% to 18%
Inter-Batch Accuracy Range (%Bias) - QCs	-6% to 6%
Inter-Batch Precision Range (%CV) - Calibrators	4% to 13%
Matrix Effect/Selectivity (Target Recovery 80-120% of Nominal) - Normal and Diseased Matrix	82-111%
Hemolyzed Sample Integrity (Target Recovery 80-120% of the Nominal)	92-116%
Dilutional Integrity	20-fold
Hook Effect	None observed up to 100 µg/mL
Benchtop Stability	4 hr at ambient temperature
Freeze-Thaw Stability	5 cycles
Interferences from Soluble TNF-alpha (1,000 pg/mL)	None detected

Figure 1. Screening Cutpoint Data (ATI).

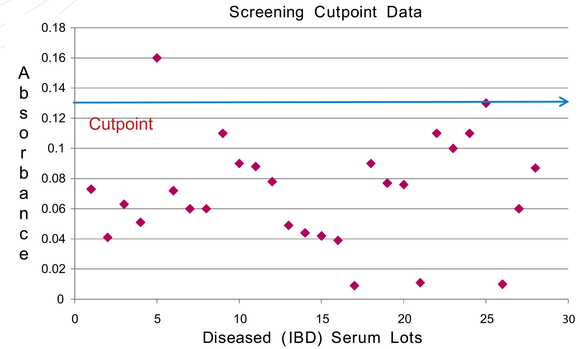

Table 5. Inter-Batch Precision of ATI.

Inter-Batch	Concentration of ATI (ng/mL)		
	NC 0	Low 250	High 500
Mean	0.023	0.157	0.342
SD	0.007	0.019	0.053
%CV	30	12	15
n	7	7	7

Table 6. Drug Tolerance Data.

Positive Control (PC)	PC Concentration (ng/mL)	IFX (ng/mL)	Response (OD)	Results (Positive/Negative)
Negative	0	0	0.042	Negative
Negative	0	5,000	0.038	Negative
Negative	0	10,000	0.029	Negative
Negative	0	20,000	0.021	Negative
Low	250	0	0.211	Positive
Low	250	5,000	0.177	Positive
Low	250	10,000	0.149	Positive
Low	250	20,000	0.071	Negative
High	500	0	0.399	Positive
High	500	5,000	0.214	Positive
High	500	10,000	0.199	Positive
High	500	20,000	0.119	Negative

Table 7. Summary ATI Validation Data.

Assay Parameter	Results
Analyte	Anti-Infliximab antibody
Positive Control	Mouse anti-Infliximab monoclonal antibody
Limit of Detection (ng/mL)	82 ng/mL
QC Concentrations (ng/mL)	250 ng/mL (Low), 500 ng/mL(High)
Intra-Batch Precision Range (%CV) - QCs	4% to 9%
Inter-Batch Precision Range (%CV) - QCs	12% to 15%
Inhibition Cut Point	41%
Matrix Effect/Selectivity - Normal and Diseased Matrix (10 lots of each) Spiked with 500 ng/mL of Positive Control	None observed
Hook Effect	None observed up to 10µg/mL
Benchtop Stability	3 hr at ambient temperature
Freeze-Thaw Stability	6 cycles
Interferences from Soluble TNF-alpha (1,000 pg/mL)	None detected
Drug Tolerance	Up to 10µg/mL

Conclusions

Pharmacovigilance is an important tool for the detection of rare characteristic differences, such as immunogenicity safety risk, between the reference product and its biosimilar. Bioanalytical assays play an important role for the reliable monitoring of serum IFX/ATI level and are critical for extracting value out of pharmacovigilance studies. The integrated ELISA developed here was found to be accurate, precise, and sensitive for the measurement of clinically relevant IFX and ATI in a diseased population to support pharmacovigilance studies. Simultaneous measurement of IFX and ATI also makes it cost-effective and easy to execute.

www.celerion.com