ALTASCIENCES

CASE STUDY

Resolving Issues with Matrix Effect

As we were developing a hybridization ELISA assay to quantitate ASOs in human feces to support a first-in-human clinical trial, issues were encountered with assay selectivity. High blank and LLOQ signals were observed in two of five individual lots, impacting selectivity, as difference in signal between lots was so high (up to fivefold) that the selectivity evaluation did not meet the acceptance criteria of 80% (see Table 2). In these initial experiments four individual lots of human feces were pooled to be used for the calibration curve and QCs.

Table 2. Nominal concentrations of ASO in individual lots unspiked or spiked at LLOQ and HQC.

Nominal ASO Concentration (ng/mL)	Blank	LLOQ		HQC	
	0.0000	0.3500		75.00	
Individual Lot #	**ASO Concentration (ng/mL)**	**ASO Concentration (ng/mL)**	**%RE**	**ASO Concentration (ng/mL)**	**%RE**
1	0.3782*	0.7640*	118.3	84.32	12.4
2	0.3665*	0.6617*	89.1	89.47	19.3
3	BLQ	0.3894	11.3	79.18	5.6
4	BLQ	0.3963	13.2	80.28	7.0
5	BLQ	0.4075	16.4	89.21	18.9

BLQ: Below Lower Limit of Quantitation (<0.3500 ng/mL)

*: % Deviation Unacceptable for QCs

Two different hypotheses were evaluated:

1. The failed evaluation is due to the nature of the matrix (feces) which may have more interference in some individual lots.

2. The method hybridization conditions are not optimal and require more optimization.

To address the first hypothesis, 12 individual lots of human feces were pooled together (instead of four) to prepare the calibration curve and QCs, and they were tested against the same five individual lots, either unspiked or spiked, at both LLOQ and HQC. Results shown in Table 3 indicate that the unspiked blank matrix from those same two lots fell below the limit of quantitation; however, the LLOQ concentrations remained above recovery. %RE was at 26.9% and 31.6% respectively for the first and second lot. We concluded that increasing the number of lots used to prepare the calibration curve and QCs was important to represent the true background of tissue sample and to reduce the nominal ASO concentration in the individual lots.

Table 3. Nominal concentrations of ASO in individual lots unspiked or spiked at LLOQ and HQC, following modification to calibration curve and QCs.

Nominal ASO Concentration (ng/mL)	Blank	LLOQ		HQC	
	0.0000	0.3500		75.00	
Individual Lot #	**ASO Concentration (ng/mL)**	**ASO Concentration (ng/mL)**	**%RE**	**ASO Concentration (ng/mL)**	**%RE**
1	BLQ	0.4440*	26.9	86.32	15.1
2	BLQ	0.4607*	31.61	84.27	12.4
3	BLQ	0.3674	5	76.98	2.6
4	BLQ	0.3691	5.5	78.24	4.3
5	BLQ	0.4129	18	87.67	16.9

BLQ: Below Lower Limit of Quantitation (<0.3500 ng/mL)

*: % Deviation Unacceptable for QCs

To further optimize the assay and better eliminate the matrix effect, the method MRD was increased and the LLOQ was raised threefold, to 1ng/g. This resulted in complete elimination of the matrix effect when the same individual selectivity lots were tested again, as illustrated in Table 4.

Table 4. Nominal concentrations of ASO in individual lots unspiked or spiked at LLOQ and HQC, after increasing the MRD and LLOQ.

Nominal ASO Concentration (ng/mL)	Blank	LLOQ		HQC	
	0.0000	1.0000		75.00	
Individual Lot #	**ASO Concentration (ng/mL)**	**ASO Concentration (ng/mL)**	**%RE**	**ASO Concentration (ng/mL)**	**%RE**
1	BLQ	1.108	10.8	80.57	7.4
2	BLQ	1.083	8.3	72.57	-3.2
3	BLQ	1.068	6.8	86.85	15.8
4	BLQ	1.198	19.8	74.82	-0.2
5	BLQ	1.073	7.3	85.12	13.5

BLQ: Below Lower Limit of Quantitation (<0.3500 ng/mL)

These results indicate that matrix interference was impacting the lower range of the assay. Creating a larger pool for calibration curve and QC, and increasing both LLOQ and MRD levels, were important to resolve the method selectivity issue.