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Mitigation of the presence of crosstalk in an ADA bridging assay using the MSD platform for the detection of antibodies against an antibody-drug conjugate

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PURPOSE

A bridging assay for the detection of anti-drug antibodies (ADAs) against an antibody-drug conjugate (ADC) was developed and validated successfully using the widely used Meso Scale Discovery (MSD) platform. This method was then used for analysis of clinical study samples during which unexpected results due to crosstalk were obtained.

METHODS

The method developed and validated for detection and characterization of antibodies against this ADC drug product was the industry standard ADA bridging multi-tiered assay using the MSD platform which included screening, titer (Figure 1) and domain characterization tiers (Figure 2). A confirmatory tier was not included due to detectable levels of pre-existing antibodies in ~90% of individuals.

Figure 1
Screening/titer tiers

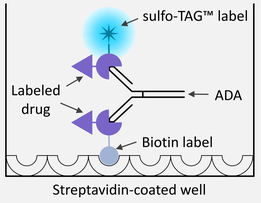
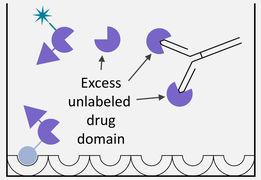
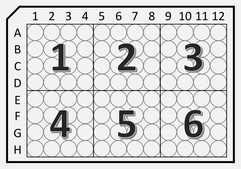


Figure 2
Characterization tier



The MSD sector imaging system takes electrochemilumiescence (ECL) readings of 96 well plates in 6 sectors of 4 by 4 wells (Figure 3).

Figure 3 MSD plate sectors



RESULTS

The validated assay was used to analyze clinical study samples for the detection and characterization of ADA against the ADC drug product. During study sample analyses, the negative control samples (tested as 3 sets per plate) occasionally generated higher than expected signals (above acceptance threshold), leading to multiple run failures. Initial investigations into the causes for this unexpected run failure rate ruled-out variability due to reagent lots, incubation steps and minimum required dilution schemes.

Further investigations focused on the presence of pre-existing ADAs observed in most human samples (79 of 90), with some reaching extremely high levels not observed during development or validation; this was due to the antibody-bacterial toxin conjugate (ADC) used in this study that included the toxin from a relatively common bacteria to which many people had already been exposed. Based on data review and high signal sample localization, a hypothesis was that very high signals in certain plate wells could affect readings in neighboring wells. This hypothesis was consistent with the fact that higher NC (negative control) signals were not observed in the titer assay tier plates where very high ADA signals were not observed (samples are always tested diluted in the titer assay).

Within every sector (refer to Figure 3), NC sample signals were correlated with the total signal of adjacent wells (R^2 = 0.35) (Figure 4) and with the total signal of the MSD plate sector (R^2 = 0.68) (Figure 5). This crosstalk phenomenon was observed if the total signal in an MSD plate sector was very high, increasing the signal of all wells in an affected sector by up to ~100 ECL counts.

Figure 4
Wells adjacent to NC sample within sector

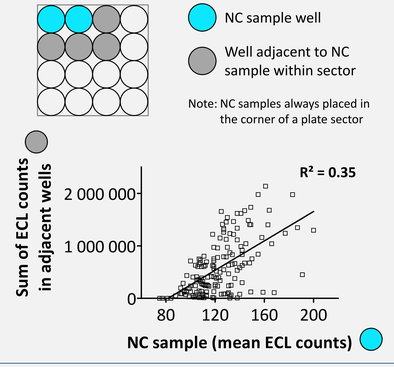
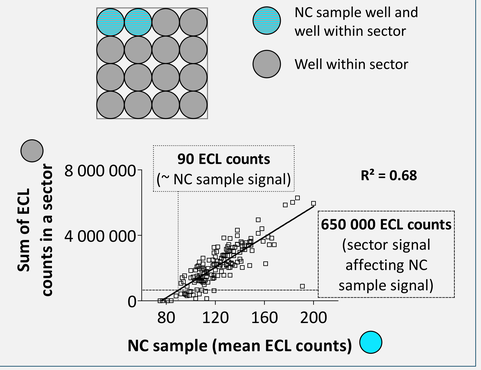


Figure 5
Wells within sector of NC sample



Simulations

In addition to effects on control samples which can lead to run failures (such as the NC sample described previously), Table 1 and Table 2 illustrate potential effects of an increase in signal of as little as 50 ECL counts on study sample results.

Table 1 Potential effects of crosstalk on study samples in the screening tier

Sample status determination	Actual ECL counts	Actual result	ECL counts + 50	Obtained result
ratio to NC < 1.30: negative ratio to NC ≥ 1.30: positive	120 (with NC at 90)	Positive (ratio to NC of 1.44)	170 (with NC at 140)	**Negative** (ratio to NC of 1.21)
	100 (with NC at 90)	Negative (ratio to NC of 1.11)	150 (with NC at 90, in sector with low ECL counts)	**Positive** (ratio to NC of 1.67)

Table 2 Potential effects of crosstalk on study samples in the characterization tiers

Sample status determination	Presence of competitor	Actual ECL counts	Actual result	ECL counts + 50	Obtained result
inhibition < 25.0%: negative inhibition ≥ 25.0%: positive	No	130	Positive (30.8% inhibition)	180	**Negative** (22.2% inhibition)
	Yes	90		140	
	No	100	Negative (10.0% inhibition)	150	**Positive** (40.0% inhibition)
	Yes	90		90 (sample in sector with low ECL counts)	

Case study

Table 3 summarizes re-analysis of samples impacted by crosstalk issues in this case study.

Table 3 Summary of results from samples reanalyzed in low ECL sectors

	Screening tier	Antibody characterization tier	Toxin characterization tier
Total number of analyzed samples	289	278	278
Number of reanalyzed samples due to sector issue	21	17	4*
Number of analyzed samples with changed status	3	4	4

*Fewer samples were reanalyzed in the toxin characterization tier as implementation of mitigation strategies (see below) had been started.

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MITIGATION

To avoid situations in which assay results are affected by very high MSD plate sector ECL counts, plate map designs should avoid including samples with projected low ECL counts (most samples) in the same plate sectors with projected very high ECL counts (undiluted post-dose study samples).

When testing unknown study samples within a plate sector that has ECL counts above a threshold of 650 000, study samples with low ECL counts in that sector would need to be repeated to eliminate the risk of false-positive/negative results.

CONCLUSIONS

Large amounts of ADAs in pre-clinical/clinical settings could lead to crosstalk issues when using the MSD platform. This phenomenon was not observed during method development and validation as these very high signals were only obtained in some study samples from treated subjects. In such cases, carefully designed assay plate maps should be considered to avoid control sample failures and to ensure accurate results in the different tiers.