Characterization of Total GIP NL-ELISA reagents

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Background

The quality and sustainability of LBAs are vital to their successful use in drug development programs. Characterization of LBA reagents therefore have a significant impact on assay reliability and reproducibility.

Characterization of critical assay reagents in the Mercodia Total GIP ELISA was an integral and essential part of developing a high-quality product that will be sustainable throughout its entire life cycle. A risk-based approach was used to identify and classify reagents that are critical for assay performance.

Conclusions

Antigen and monoclonal antibodies used in Mercodia Total GIP NL-ELISA are well characterized.

The antigen is stable, has a high purity, known identity and concentration and a good similarity with endogenous GIP in the assay.

Capture and detection antibodies have known concentrations and high affinity for GIP (3-42) and GIP (1-42).

Results

Critical reagents classified with high-risk in Mercodia Total GIP NL-ELISA were antigen and antibodies. Classification of reagents in risk categories were determined during the assay development and is subsequently continuously monitored via post market surveillance of the product.

Antigen

Table 1. Antigen concentrations were determined with quantitative amino acid analysis and recovery was calculated based on the expected concentrations. Synthetic human GIP (3-42) was chosen as reference material in the calibrators since that form has the highest prevalence in samples with endogenous GIP.

Sample	Analysis 1 (µg/µL)	Analysis 2 (µg/µL)	Analysis 3 (µg/µL)	Average (µg/µL)	Expected (µg/µL)	Recovery (%)
GIP (3-42)	0.71	0.71	0.75	**0.72**	0.78	93.0
GIP (1-42)	0.84	0.83	0.83	**0.83**	0.86	96.8

Table 2. Obtained results from the antigen identity and purity analysis obtained by HPLC-UV LC-MS/MS analysis.

Sample	Measured mass (Da)	Theoretical mass (Da)	Delta mass (Da)	Sequence coverage (%)
GIP (3-42)	4746.33	4746.37	-0.04	**100**
GIP (1-42)	4980.42	4980.47	-0.05	**100**

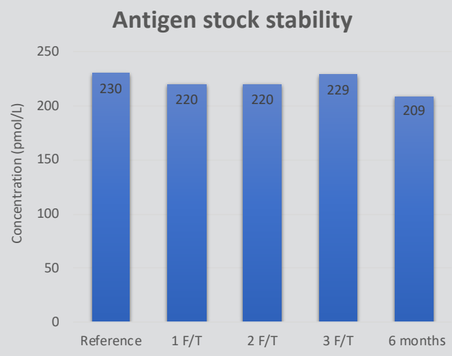

Figure 1. Antigen stock stability for three freeze/thaw cycles and 6-months storage in -80°C. Long-term stability is continuously monitored in real time.

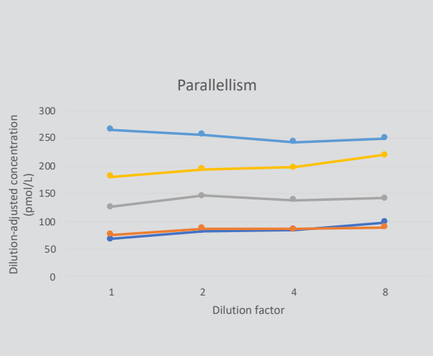

Figure 2. Parallelism in 5 plasma samples showing good similarity between the synthetic GIP (3-42) in the calibrators and endogenous GIP in samples.

Antibodies

Concentration of capturing antibody and detection antibody was determined using absorbance measurements (A280).

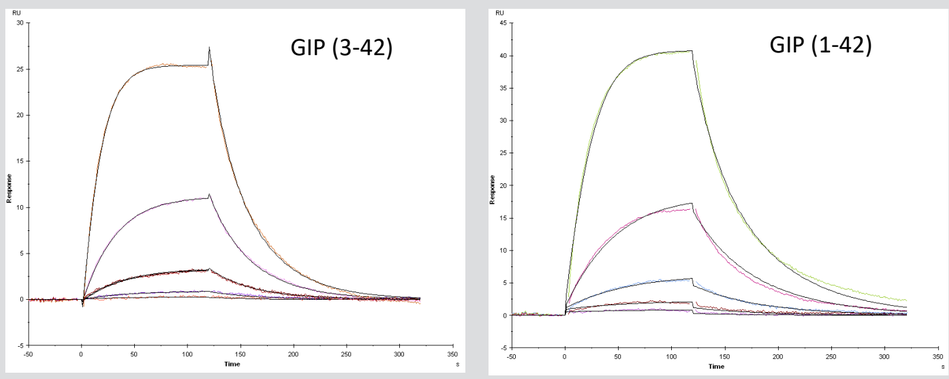

Figure 2. Sensorgrams from the SPR analysis showing binding between the detection antibody and GIP (3-42 and 1-42, respectively) at concentrations ranging from 0.39 to 100 ng/mL.

Table 3. Kinetic and affinity values for the detection antibody and GIP obtained from the SPR analysis.

Sample	ka (1/Ms)	kd (1/s)	KD (nM)
GIP (3-42)	$2.9x10^6$	0.045	17
GIP (1-42)	$3.0x10^6$	0.046	16

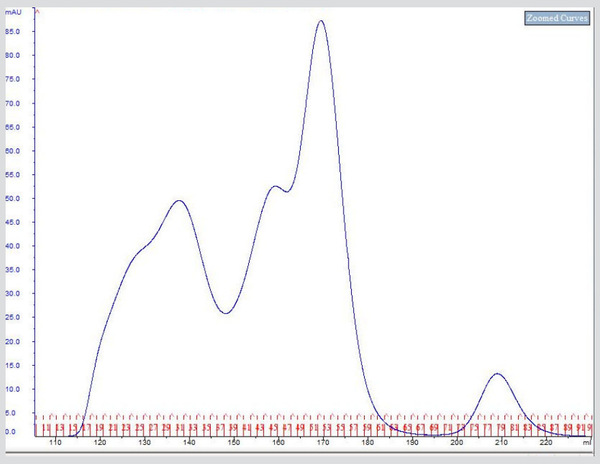

Figure 3. Chromatogram for HRP-conjugated detection antibody used in Mercodia Total GIP NL-ELISA. Fractions which mainly include the single conjugated antibodies were chosen.

Methods

Characterization of the critical components involved analysis of concentration, purity, identity and stability. It was performed by quantitative amino acid analysis, absorbance measurements and HPLC-UV LC-MS/MS analysis for the antigen and SPR, absorbance measurements and size-exclusion chromatography for the antibodies.

References

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