# DESIGN OF BIOMARKER VALIDATION FOR INTENDED USE IN DRUG DEVELOPMENT - VALIDATION OF A HIGHLY SPECIFIC ENZYME-LINKED IMMUNOSORBENT ASSAY FOR INSULIN IN RELATION TO FDA, EMA AND CLSI GUIDELINES

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## AIM

The aim of this study was to re-validate a well-established ELISA for Insulin, available on the market for more than 20 years, for intended use in drug development programs in relation to FDA, EMA and CLSI guidelines.

## BACKGROUND

Insulin is often used as a pivotal biomarker in all phases of drug development. Validated methods for Insulin measurement is needed. The use and/or development of different insulin analogs pose a special challenge of specificity. The design of biomarker validation in relation to FDA[1] and EMA[2] guidelines is a challenge, given the aim of the guidelines is mainly to guide pharmacokinetic (PK) studies. Moreover, native insulin or insulin analogs is present in all donor matrices.

## METHODS

Validation of Insulin ELISA was performed with a fit for purpose approach using validation guidelines for PK assays as a starting point. The validation results using EMA/FDA guidelines was compared to the result following CLSI recommendations. Special consideration had to be taken to determine nominal values of Insulin concentration in the samples, and to treat sample specific deviations.

## RESULTS

**Intra- and inter precision** for five quality control (QC) samples (LLOQ, low, medium, high and ULOQ), expressed as coefficient of variation CV (%), analyzed in four replicates in 12 runs is shown in Figure 1. **Accuracy** calculated versus the nominal concentration (i.e. the spiking level of the QC samples) and the total error calculated as the sum of the relative error and the CV percentages is also shown.

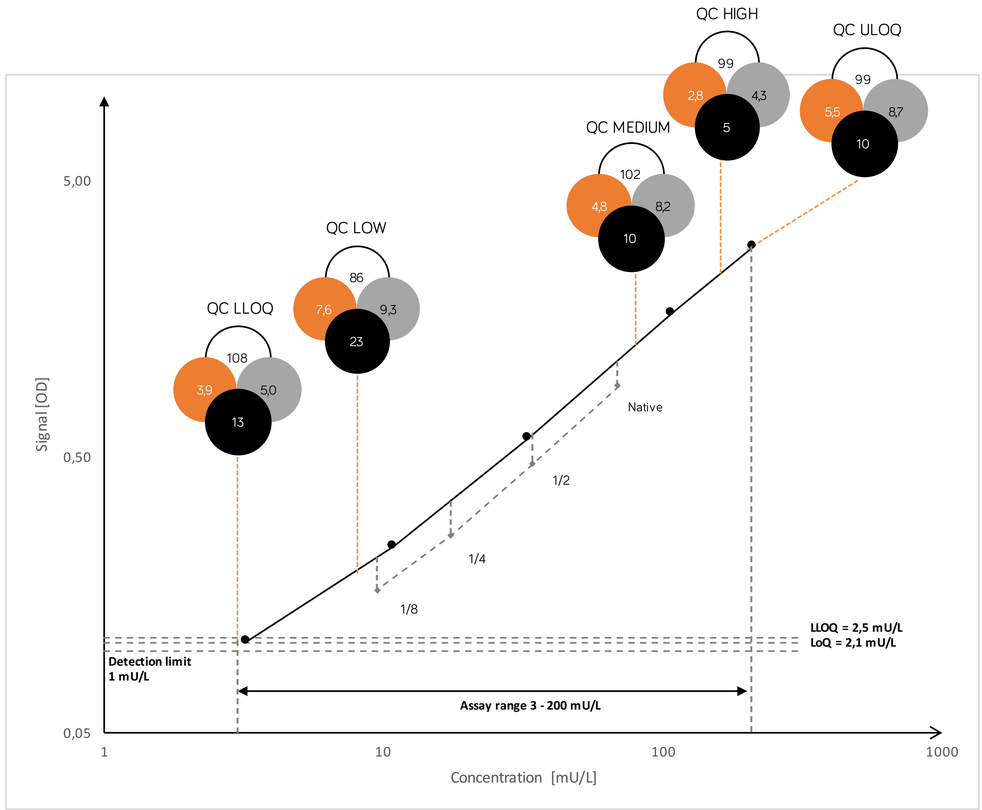


**Figure 1**. Validation results

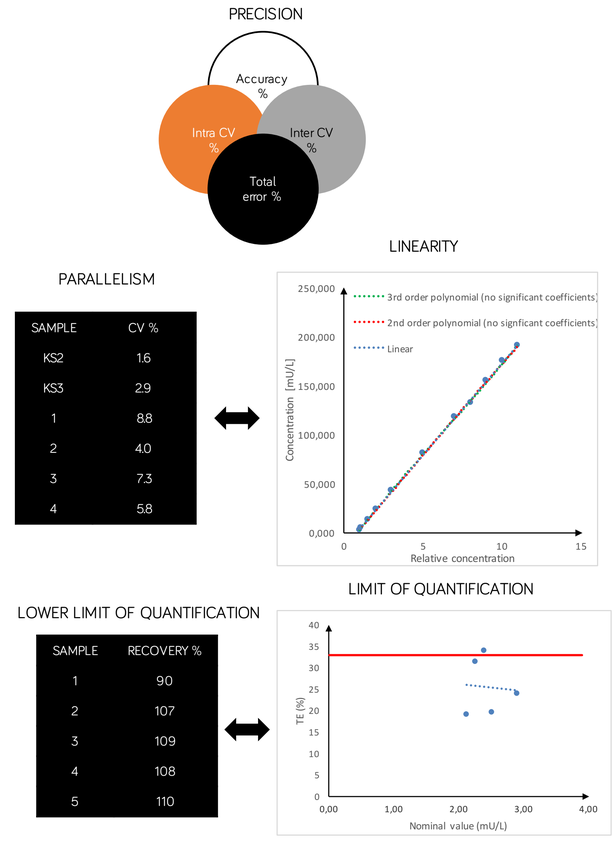


**Table 1**. Interference

| | Concentration range [pM] | Recovery [%] LLOQ | Recovery [%] ULOQ |
|---|---|---|---|
| C-peptide | 1000 – 4000 | 105 - 112 | 100 - 94 |
| Proinsulin | 32.5 – 130 | 110 - 117 | 101 - 101 |
| Insulin Aspart | 1500 – 6000 | 147 - 1048 | 102 - 121 |
| Insulin Glargine | 690 – 2760 | 531 - 4405 | 107 - 122 |
| Insulin Detemir | 9000 – 36 000 | 102 - 85 | 96 - 69 |
| Insulin Glulisine | 18 000 – 72 000 | 92 - <71 | 81 - 56 |
| Insulin Lispro | 18 000 – 72 000 | 95 - <71 | 89 – 52 |
| Insulin Degludec | 9 000 – 36 000 | 98 - 91 | 91 – 76 |

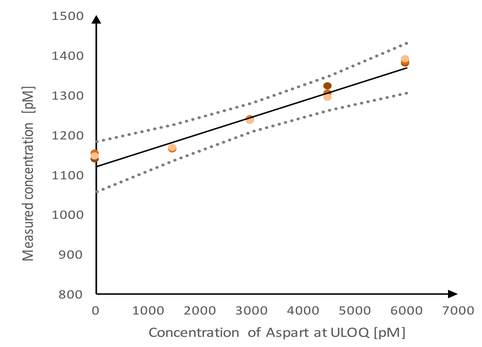


**Figure 2**. Dose-response curve of Aspart at ULOQ concentration

The result of **Parallelism** for two serum controls and four samples diluted in three steps with the dilution factor 2 in each step with buffer as the diluent is shown in Figure 1. As a comparison **Linearity**[3], for 11 different concentration levels prepared by mixing different fractions of a high serum and a low serum of Insulin is shown. Regression analysis of the concentration versus the relative concentration of the concentration levels was performed using linear, second order and third order polynomial and the significance of the coefficient was calculated.

To verify that samples at a concentration of Cal 1 can be measured accurately and precisely, **LLOQ** was determined for five serum samples diluted to a concentration of 2.5 mU/L with buffer in six runs and recovery values were calculated (Figure 1). The nominal value was calculated with respect to the dilution factor by measuring the concentration of the reference sample in six repetitive runs. Evaluation of **LoQ**[4] was performed for the same samples in four runs. LoQ is determined to 2.1 mU/L (the lowest concentration that meets the acceptance criteria) and LLOQ is determined to 2.5 mU/L (average concentration of five samples).

Recovery values for evaluation of **Specificity** for five dilutions of the potentially cross-reacting substances in serum samples at LLOQ and ULOQ is shown in Table 1. Recovery values were calculated versus the determined concentration of Insulin at LLOQ/ULOQ. The same set of samples as described above were also evaluated for **Interference**[5] using a paired difference test, and for substances that were determined to interfere further evaluation of the dose-response relationship was performed, see examples of Aspart at ULOQ concentration of Insulin in Figure 2.

**Table 2**. Determined limits for acceptance according to EMA/FDA and CLSI regulations.

| | Limits for acceptance (EMA/FDA) | Determined Limits for acceptance (CLSI) |
|---|---|---|
| Intra Precision | CV < 20 % (25 % at LLOQ)[1,2] | - |
| Inter Precision | CV < 20 % (25 % at LLOQ)[1,2] | - |
| Accuracy | Recovery 100 ± 20 % (100 ± 25 % at LLOQ)[1,2] | - |
| Total error (TE) | TE < 30 % (40 % at LLOQ and ULOQ)[2] | - |
| Specificity/Interference | Recovery 100 ± 25 %[2] | $H_0$ (null hypothesis) = 100 ± 25 % |
| Parallelism/Linearity | CV < 30 %[2] | Deviation from linearity < 20 % |
| LLOQ/LoQ | Recovery 100 ± 25 %[1,2] | TE % < 32.9 %[3] |

## CONCLUSIONS

Mercodia Insulin ELISA fulfills the requirements for precision, accuracy, sensitivity, selectivity and specificity. The precision and accuracy of the assay is very high, and LLOQ is set to 2.5 mU/L (LoQ = 2.1 mU/L).

The assay is linear in the measuring range between 3 – 200 mU/L. The Insulin analogs Aspart, Degludec, Glulisine, Lispro, Detemir do not interfere at physiological concentrations. Possible interference of Insulin analogs at supraphysiological concentrations has been investigated. Well defined concentration levels where each Insulin analog shows interference has been established.

Depending on the determined limits for acceptance according to CLSI, slightly different validation parameters are obtained using the different guidelines, but in overall the same conclusions of the assay performance can be drawn.

### References

1. Guidance for Industry, Bioanalytical Method Validation, Draft Guidance, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine, September 2013, Biopharmaceutics, Revision 1
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6. Westgard Desirable Biological Variation Database, 2014

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