VALIDATION OF A NOVEL INSULIN **LISPRO ELISA**

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PURPOSE

Insulin lispro is a rapid-acting insulin analog that was first introduced in 1996[1] to treat people with diabetes.

For an LBA (ligand binding assay) to qualify as a PK assay, several requirements for assay design, as well as analytical performance, are specified in industry guidelines[2,3]. For insulin lispro, an easy-to-use, high quality PK assay that can meet the pharmaceutical industry requirements for multiple phases of drug development is needed. In addition, differentiating lispro not only from other rapid- and long-acting insulin analogs, but also from native insulin, is a need that demands careful method development.

CONCLUSIONS

This novel PK assay, Mercodia Lispro NL-ELISA is both sensitive and specific for determination of insulin lispro. With pre-diluted calibrators in serum matrix at six levels, as evidenced by the robust assay performance characteristics, this method is well suited for multiple phases of drug development and meets the requirements of EMA[3] and FDA[2] validation.

- Optimized for pharma use
- High specificity to lispro
- Sample volume: 10 µL
- Measuring range: 1-500 mU/L
- Results in 2h 15 min
- Detection method: Chemiluminescence
- Validated for use in human and non-diabetic or diabetic pig models
- No pre-treatment or dilution of samples
- No interference from to insulin autoantibodies

RESULTS

The Lispro NL-ELISA has been fully validated based on EMA[3] and FDA[2] regulatory guidelines. Results from the validation study are presented below.

PARALLELISM, HUMAN AND PORCINE SAMPLES

Validation of samples from human serum/plasma and porcine plasma, showed that all samples were parallel to the serum calibrator curve (Figure 1).

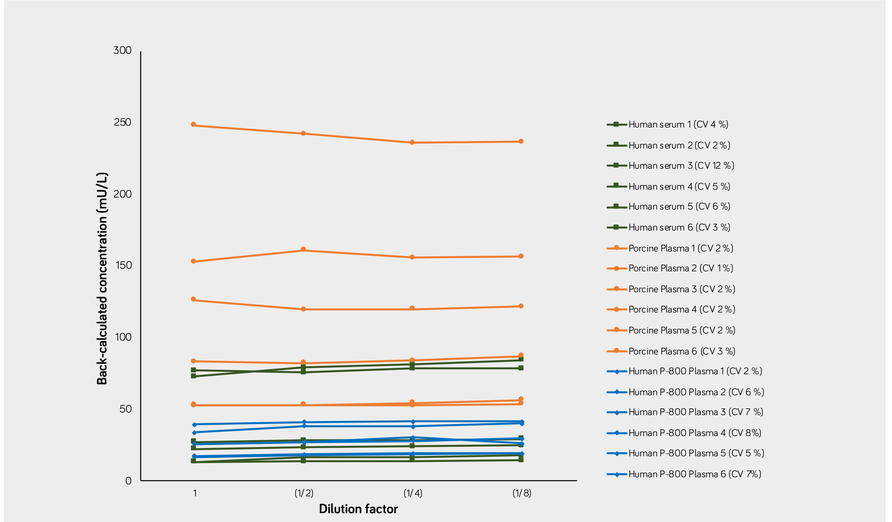

Figure 1 Parallelism. a) Samples were serially diluted (1/2), (1/4) and (1/8) and precision between back-calculated concentration of samples were calculated. Acceptance criteria was set to CV < 30% according to EMA[3] guidelines.

MEASURING RANGE

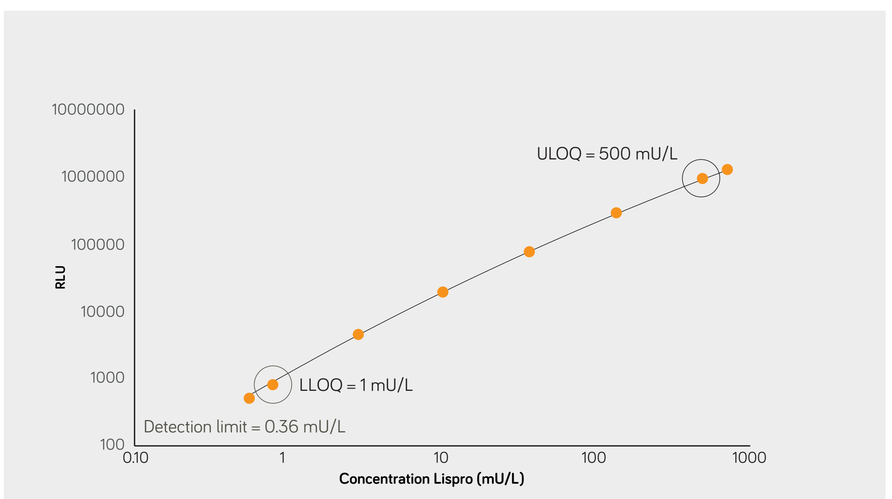

Figure 2: The assay employs chemiluminescent detection to achieve a broad dynamic measuring range. LLOQ and ULOQ were determined according to regulatory guidelines (EMA[3]/FDA[2]). The assay is calibrated with ready to use, lyophilized serum calibrators. Serum based anchor points below Calibrator 1 and above Calibrator 6 are included in the kit.

SPECIFICITY

Specificity to insulin lispro without cross-reactivity to native insulin, native proinsulin, or any of the most commonly used insulin analogs was observed (Table 1).

TABLE 1. Specificity

Substance	Concentration intervall	Cross-reactivity*	Interference Acceptence criteria 100 ± 25 % LLOQ (Recovery %)	Interference Acceptence criteria 100 ± 25 % ULOQ (Recovery %)
Native Human Insulin	50-400 mU/L	N.D	105-112 %	97-114 %
Native Human Proinsulin	50-300 pmol/L	N.D	104-113 %	103-110 %
Glargine	50-600 mU/L	N.D	96-117 %	89-107 %
Glargine M1	50-600 mU/L	N.D	98-105 %	100-107 %
Glargine M2	50-600 mU/L	N.D	104-123 %	109-115 %
Degludec	50-600 mU/L	N.D	101-113 %	103-109 %
Detemir	50-600 mU/L	N.D	107-116 %	108-110 %
Insulin NHP	50-600 mU/L	N.D	106-116 %	102 - 108 %
Aspart	50-600 mU/L	N.D	95-111 %	101 - 106 %
Glulisine	50-600 mU/L	N.D	101-112 %	99-103 %

***N.D=** Not detected

SELECTIVITY

Selectivity in type 1 diabetes (T1D) with verified levels of insulin auto-antibodies showed that T1D samples can be run directly in Mercodia Lispro NL-ELISA without pre-treatment (Table 2).

TABLE 2. Selectivity in samples containing auto-insulin antibodies

	Interference at 2 mU/L Lispro (Recovery %) Acceptance criteria 100 ± 25 %	Interference at 50 mU/L Lispro (Recovery %) Acceptance criteria 100 ± 20 %	Insulin autoantibody levels in sample (U/ml)
T1D sample 1	113	92	6.3
T1D sample 2	117	90	8.3
T1D sample 3	110	96	13.2
T1D sample 4	94	90	9.6

PRECISION AND ACCURACY

Precision and accuracy were evaluated by spiking serum samples with different levels of insulin lispro, Humalog (Eli Lilly and Company). LLOQ was established at 1 mU/L and ULOQ at 500 mU/L, according to EMA[3]/FDA[2] guidelines (Table 3).

TABLE 3. Precision and accuracy

Control	Repetability (CV %)	Within Laboratory Precision (CV %)	Within-run accuracy % Acceptance criteria 100 ±20%, 100 ± 25% LLOQ/ULOQ	Between-run accuracy % Acceptance criteria 100 ±20%, 100 ± 25% LLOQ/ULOQ	Total error (TE) % Acceptance criteria TE<30%, TE <40% LLOQ/ULOQ
Human Serum Control LLOQ (1 mU/L)	6.7	7.7	80-101	94	14
Human Serum Control Low (2 mU/L)	3.5	5.8	82-100	95	11
Human Serum Control Medium (25 mU/L)	2.2	3.7	80-88	86	18
Human Serum Control High (400 mU/L)	1.9	3.0	87-95	91	12
Human Serum Control ULOQ (500 mU/L)	2.5	3.4	86-96	92	12

*12 runs, 2 Laboratory technicians, 4 days

REFERENCES

1. "Drugs@FDA:FDA Approved Products: Humalog". U.S. Food and Drug Administration. Retrieved 25 June 2018.
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3. Guideline on bioanalytical method validation, European Medicines Agency (EMA), Science Medicines Health, 21 July 2011.

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