

## Cross-reactivity of human insulin analogs in the Insulin ELISA, the Ultrasensitive Insulin ELISA and the Iso-Insulin

Mercodia Insulin ELISA (10-1113-01/10) and Mercodia Ultrasensitive Insulin ELISA (10-1132-01) are two insulin ELISAs with monoclonal antibodies that do not cross-react with insulin detemir, insulin aspart, insulin glulisine or insulin lispro. For Mercodia Insulin ELISA, an alternative sequence protocol (see Technical Note 34-0143) may be used that do not detect insulin glargine or glargine metabolites M1 or M2. By using an insulin assay that does not detect insulin analogs, it is possible to detect the contribution of an insulin analog to the free insulin profile in an individual. The specific detection of endogenous insulin in Mercodia Insulin ELISA or Mercodia Ultrasensitive Insulin ELISA may be combined with insulin analog detection in Mercodia Iso-Insulin ELISA (10-1128-01), that cross-react with insulin aspart, detemir, glargine, glulisine and lispro.

By combining Mercodia Iso-Insulin ELISA and Mercodia Insulin ELISA, Lindström *et al.* examined the pharmacokinetics of insulin aspart and lispro in 13 patients with type 1 diabetes after a 10-unit subcutaneous injection of the analogs (Lindström *et al.* 2002).

Heald *et al.* used Mercodia Iso-Insulin ELISA in an insulin regimen compliance study in a clinical case presenting with type 1 diabetes. Measurement of insulin detemir and aspart in Mercodia Iso-Insulin ELISA confirmed that the patient showed compliance with insulin regimen (Heald *et al.* 2006).

In the reference list at the end of this technical note additional articles are included where Mercodia insulin ELISAs have been used to detect human insulin and analogs.

### Cross-reactivity of human insulin analogs in Mercodia insulin ELISAs:

We have specifically determined the cross-reactivity of aspart (NovoRapid®, Novo Nordisk), detemir (Levemir®, Novo Nordisk), glargine (Lantus®, Aventis Pharma), glulisine (Apidra®, Aventis Pharma) and lispro (Humalog®, Lilly) in Insulin ELISA (10-1113-01), Ultrasensitive Insulin ELISA (10-1132-01) and Iso-Insulin ELISA (10-1128-01). Glargine metabolites M1 and M2 were kindly provided by R&D DSAR/Biomarkers, Bioim.&Biol.Ass. FF, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany (Carlsson *et al.* 2012).

For conversion of insulin concentrations, the following conversion factors were used:  
1 µg/L = 23 mU/L; 1 mU/L = 6.0 pmol/L

Summary of results:

Cross-reactivity in Insulin ELISA (10-1113-01/10):

Insulin analog	Mean cross-reaction	Range cross-reaction	Range tested conc.
Insulin aspart	4.0 %	1.3 – 7.7 %	30.5 – 97.7 µg/L 702 – 2 247 mU/L 4 209 – 13 482 pmol/L
Insulin detemir	<0,0000009 %	-	14 200 000 µg/L* 326 600 000 mU/L* 1 959 600 000 pmol/L*
Insulin glargine	24 %**	10 – 39 %	2.50 – 20.0 µg/L 57.5 – 460 mU/L 345 – 2760 pmol/L
Insulin glulisine	<0,000004 %	-	3 490 000 µg/L* 80 270 000 mU/L* 481 620 000 pmol/L*
Insulin lispro	<0,000004 %	-	3 500 000 µg/L* 80 500 000 mU/L* 483 000 000 pmol/L*

\* Highest concentration tested.

\*\* For Mercodia Insulin ELISA, an alternative sequence protocol (see Technical Note 34-0143) may be used that do not detect insulin glargine or glargine metabolites M1 or M2.

Cross-reactivity in Ultrasensitive Insulin ELISA (10-1132-01):

Insulin analog	Mean cross-reaction	Range cross-reaction	Range tested conc.
Insulin aspart	3.0 %	0.8 – 8.1 %	1.78 – 10.0 µg/L 40.9 – 230 mU/L 246 – 1 380 pmol/L
Insulin detemir	<0,0000006 %	-	14 200 000 µg/L* 326 600 000 mU/L* 1 959 600 000 pmol/L*
Insulin glargine	12 %	7.2 – 23 %	0.114 – 1.82 µg/L 2.62 – 41.9 mU/L 15.7 – 251 pmol/L
Insulin glulisine	<0,0000002 %	-	3 490 000 µg/L* 80 270 000 mU/L* 481 620 000 pmol/L*
Insulin lispro	<0,0000004 %	-	3 500 000 µg/L* 80 500 000 mU/L* 483 000 000 pmol/L*

\* Highest concentration tested.

Cross-reactivity in Iso-Insulin ELISA (10-1128-01):

Insulin analog	Mean cross-reaction	Range cross-reaction	Range tested conc.
Insulin aspart	100 %	92 - 115 %	0.153 - 2.50 µg/L 3.52 - 57.5 mU/L 21.1 - 345 pmol/L
Insulin detemir	28 %	24 - 34 %	0.610 - 10.0 µg/L 14.0 - 230 mU/L 84.2 - 1380 pmol/L
Insulin glargine	58 %	47 - 70 %	0.610 - 5.00 µg/L 14.0 - 115 mU/L 84.2 - 690 pmol/L
Insulin glargine M1	47 %	32 - 55 %	0.313 - 5.00 µg/L 7.19 - 125 mU/L 53.1 - 870 pmol/L
Insulin glargine M2	32 %	21 - 46 %	0.313 - 5.00 µg/L 7.19 - 125 mU/L 53.1 - 870 pmol/L
Insulin glulisine	123 %	92-136 %	0.218-3.49 µg/L 5.01-80.3 mU/L 30.1-482 pmol/L
Insulin lispro	112 %	93 - 145 %	0.153-2.50 µg/L 3.52-57.5 mU/L 21.1 - 345 pmol/L

### Concluding remarks

#### Mercodia Insulin ELISA (10-1113-01/10)

- Insulin detemir, insulin glulisine and insulin lispro do not cross-react in the Insulin ELISA.
- For insulin aspart, with a mean cross-reactivity of 4.0 % in the Insulin ELISA, the lowest detectable concentration (of insulin aspart) is 230 mU/L in this assay.
- For insulin glargine, with a mean cross-reactivity of 24 % in the Insulin ELISA, the lowest detectable concentration in this assay is  $\geq 30$  mU/L\*\*.

\*\* For Mercodia Insulin ELISA, an alternative sequence protocol (see Technical note 34-143) may be used that do not detect insulin glargine or glargine metabolites M1 or M2.

#### Mercodia Ultrasensitive Insulin ELISA (10-1132-01)

- Insulin detemir, insulin glulisine and insulin lispro do not cross-react in the Ultrasensitive Insulin ELISA.
- For insulin aspart, with a mean cross-reactivity of 3.0 % in the Ultrasensitive Insulin ELISA, the lowest detectable concentration of insulin aspart is  $\geq 19$  mU/L in this assay.
- For insulin glargine, with a mean cross-reactivity of 12 % in the Ultrasensitive Insulin ELISA, the lowest detectable concentration in this assay is  $\geq 2.1$  mU/L.

#### Mercodia Iso-Insulin ELISA (10-1128-01)

- In the Iso-Insulin ELISA, all five analogs (aspart, detemir, glargine, glulisine and lispro) cross-react to various degrees.
- For insulin aspart, with a mean cross-reactivity of 100 % in the Iso-Insulin ELISA, the lowest detectable concentration is  $\geq 3$  mU/L in this assay.
- For insulin detemir, with a mean cross-reactivity of 28 % in the Iso-Insulin ELISA, the lowest detectable concentration is  $\geq 11$  mU/L in this assay.
- For insulin glargine, with a mean cross-reactivity of 58 % in the Iso-Insulin ELISA, the lowest detectable concentration is  $\geq 6$  mU/L in this assay.
- For insulin glargine M1, with a mean cross-reactivity of 47 % in the Iso-Insulin ELISA, the lowest detectable concentration is  $\geq 9$  mU/L in this assay.
- For insulin glargine M2, with a mean cross-reactivity of 32 % in the Iso-Insulin ELISA, the lowest detectable concentration is  $\geq 14$  mU/L in this assay.
- For insulin glulisine, with a mean cross-reactivity of 123 % in the Iso-Insulin ELISA, the lowest detectable concentration is  $\geq 3$  mU/L in this assay.
- For insulin lispro, with a mean cross-reactivity of 112 % in the Iso-Insulin ELISA, the lowest detectable concentration is  $\geq 3$  mU/L in this assay.

## References

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