Mercodia

Technical Note, No: 34-0115, v.5.0

Specificity of the Mercodia Proinsulin ELISA

The Mercodia Proinsulin ELISA, 10-1118-01, is specific for human proinsulin and detects both the split and the des metabolites.

Specificity data

The cross reactivity of the metabolites des (64,65) and split (65,66) in the Mercodia Proinsulin ELISA has little clinical or analytical relevance as these circulate at low concentrations relative to other forms of proinsulin.

Table 1. Cross reactivity in Mercodia Proinsulin ELISA (10-1118-01).

Insulin	<0.03 %
C-peptide	<0.006 %
Proinsulin Des (64–65)	84 %
Proinsulin Split (65–66)	90 %
Proinsulin Des (31–32)	95 %
Proinsulin Split (32–33)	95 %

Processing of human proinsulin

Human Proinsulin (hPI) is processed by endoproteolysis at the junctions of amino acids (32,33) and (65,66) to give split (32,33) hPI and split (65,66) hPI. The exposed, C-terminal, basic amino acids in the split metabolites are removed by carboxypeptidase to yield des (31,32) and des (64,65).

Concentrations of different proinsulin derivatives in serum

Sobey *et al.* constructed specific two-site assays for intact hPI, insulin, split (65-66) hPI and split (32-33) hPI. By using the different assays, they found < 1.0 pM of split (65-66) hPI after overnight fasting in eight normal male subjects and the maximum individual concentration reached in plasma taken during an oral glucose tolerance test was 3.8 pM. Intact proinsulin was 2.3±0.3 pM and split (32-33) proinsulin was 2.1±0.7 pM in the fasting state of the eight normal subjects and 9.9±1.4 pM and 19.7±6.0 pM respectively, during the oral glucose tolerance test.

The authors conclude that the very low concentration of split (65-66) hPI meant that this derivative did not interfere significantly with the specificity of the assays of intact proinsulin and insulin used in the study. The authors claim that ”in no system was any difference detected between the behavior of (65-66) split and des (64-65) human proinsulin, nor between (32-33) split and des (31-32) human proinsulin”.

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In studies examining intact and split proinsulin in human subjects only intact and split (32,33) hPI is quantified. The circulating concentrations of split (32,33) hPI is usually found to be as high or higher than intact hPI (Wareham *et al.*, Zethelius *et al.*)

Clinical implication

It is generally believed that increased proinsulin concentrations are associated with risk of coronary heart disease, insulin resistance and development of type 2 diabetes. However, results are contradictive regarding the importance of split (32,33) hPI and intact hPI versus total proinsulin in prediction of disease.

The cross reactivity of the metabolites des (64,65) and split (65,66) in the Mercodia Proinsulin ELISA has little clinical or analytical relevance as these circulate at low concentrations relative to other forms of proinsulin.

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