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Wood, W. G., C. J. Strasburger, H. Fricke, A. Gadow
and P. C. Scriba

**SPALT – Solid Phase Antigen Luminescence Technique:
An Alternative to Radioimmunoassay for Serum
Insulin Determinations**

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Previous alternatives to radioimmunoassay have almost always come up against difficulties when proteo-hormone assays, sensitive enough for routine purposes have had to be developed. The present abstract describes the use of the SPALT method for the assay of serum insulin concentrations.

The assay uses an immobilised antigen, either bovine or porcine insulin, covalently bound to a solid phase, here activated microcrystalline cellulose. The label used is a

pyruvate kinase second antibody conjugate, here donkey-anti-guinea pig IgG. By altering the label to a rabbit-anti-human IgG, the same system could be used for the detection and quantification of antibodies to either bovine or porcine insulin.

The assay is carried out as follows:

The first incubation between serum and first antibody is performed exactly as in a radioimmunoassay with "cold" preincubation. An excess of solid-phase antigen is then added to react with the free antibody. After washing and centrifuging followed by removal of the supernatant, the solid phase is incubated with pyruvate kinase labelled second antibody. The wash step is then repeated before the generation of ATP from ADP and phosphoenol pyruvate is followed in the luminometer.

Full details of the light generation and measurement steps have been published (1). Assays with a total incubation time between 6 and 72 hours have been studied. The working range of the assays was ca. 5–200 mU/l insulin using a 50 or 100 µl serum sample.

Precision of the method is comparable with radioimmunoassay, intra-assay coefficients of variation under 5 % being obtained.

Reference

1. Fricke, H., Strasburger, C. J. & Wood, W. G. (1982) *This J.* **20**, 91–94.

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