

Measurement of Insulin Immunoreactivity in Human Plasma and Serum

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Featured article: Andersen L, Dinesen B, Jørgensen PN, Poulsen F, Røder ME. Enzyme immunoassay for intact human insulin in serum or plasma. *Clin Chem* 1993;39:578–82.⁴

Our 1993 report described a monoclonal antibody-based ELISA for insulin that had greater specificity than existing RIAs. This specificity allowed determination of intact insulin only, without comeasurement of proinsulin and the conversion intermediate des(31,32)-proinsulin. In patients with type 2 diabetes or with high concentrations of insulin precursors in the blood, the assay measured intact insulin immunoreactivity from plasma and serum samples. The assay was also suitable for large-scale studies.

Immunochemical methods for estimating insulin concentrations have been used for 50 years. In 1959 Berson and Yalow introduced the RIA method for the determination of insulin (1), which led to a Nobel Prize in 1977. Competitive insulin RIAs based on polyclonal antibodies had many limitations, however, including limited stability of the radioactive isotope, limited amounts of the antibodies, and unspecific determination of insulin precursors and insulin degradation products together with insulin. The assays were laborious for large-scale use. The discovery of monoclonal antibodies made it possible to produce potentially unlimited amounts of antibodies directed toward selected epitopes on the insulin molecule. In 1989 Sobey and coauthors confirmed that the use of monoclonal antibodies with predefined specificities in a 2-site immunoassay design yielded more specific assays against human insulin and proinsulin conversion intermediates (2).

In the early 1990s the various methods used for quantification of insulin immunoreactivity were not standardized (3). This situation was attributable not

only to different specificities of the assays, but also to assay characteristics such as linearity, recovery, and accuracy. As revealed by the American Diabetes Association Task Force Report (4), various assays used to measure insulin in identical serum and plasma samples produced widely disparate results. To address this problem the Task Force proposed a 3-step procedure that encompassed internal assay performance, external comparability, and a recertification process.

The need for improved standardization, precision, and specificity was evident. Large-scale epidemiological studies were performed to demonstrate the role of insulin in diabetes susceptibility and cardiovascular risk. Differential roles were hypothesized for the relationships between insulin and proinsulin and its conversion intermediates as biomarkers in the prediabetic state and as cardiovascular risk factors in individuals with and without diabetes.

We characterized various monoclonal antibodies with epitope specificities for different parts of the A and B chains of human insulin and of the cleavage sites of proinsulin (AC and BC cleavage sites). Our aim was to produce 2-site sandwich assays with specificities toward intact human insulin, intact human proinsulin, and the 2 proinsulin conversion intermediates [des(31,32)- and des(64,65)-proinsulin]. We used a solid-phase ELISA with a 96-well plate format. We succeeded with assays for intact proinsulin and intact insulin. The insulin assay was based on 2 monoclonal antibodies, OXI-005 (epitope near the C-terminal of the B-chain) and HUI-018 (epitope A-loop). The observed specificity of the assay was surprising, with immunoreactivity toward intact insulin but also to a lesser degree toward split (65,65)- and des(65,65)-proinsulin. Thus, the assay was in theory not specific for intact insulin. It turned out to be clinically specific, however, because it has now been shown by use of a different method (HPLC) that these 2 proinsulin intermediates are present only in tiny amounts in the circulation.

This characterization showed that the assay was clinically relevant and that it fulfilled the requirements for a high-throughput, rapid, and analytically sensitive insulin assay. These properties may explain why the assay has been used for more than 20 years. Other insulin assays have been developed (e.g., the electrochemiluminescence insulin assay from Roche Diag-

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⁴ This paper has been cited more than 350 times since publication.

nostics and the microparticle enzyme immunoassay from Abbott), but neither of these assays has been “groundbreaking” from a clinical point of view. The antibodies from our original ELISA are constituents of a commercial ELISA kit (Dako Cytomation) and a DELFIA kit (PerkinElmer).

The requirement of insulin measurement for drug development in the pharmaceutical industry has made new assays necessary. A new homogenous insulin immunoassay has been developed that is based on luminescence oxygen channeling in a 384-well format that uses the antibodies HUI-018 and OXI-005 (5). In industrial applications this assay has been demonstrated to have performance superior to both the original ELISA and to several other immunoassays, and it may also have advantages within clinical chemistry.

Future demand for measurement of insulin immunoreactivity may involve both clinical applications and pharmaceutical development. The homeostatic model assessment for estimation of insulin sensitivity and β -cell function requires insulin analyses and will likely be used in epidemiological studies and phase III–IV studies of new drugs for type 2 diabetes. Furthermore, pharmacokinetic studies of new generations of insulin analogs will require specific measurements of these molecules.

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