

Soeldner J S & Slone D. Critical variables in the radioimmunoassay of serum insulin using the double antibody technic. *Diabetes* 14:771-9, 1965.

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Although a double antibody radioimmunoassay method for serum insulin had been previously proposed, the design of an accurate and reliable method had been elusive. This was achieved by paying careful attention to the duration of time after adding the second antibody to the system to insure adequate precipitation and partitioning of the bound from the free insulin. [The SC® indicates that this paper has been cited over 380 times since 1965.]

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"Although the original radioimmunoassay for insulin had been described in the late 1950s, and a double antibody technique described in the early 1960s by Morgan and Lazarow,¹ the assay simply wasn't accurate or reliable enough for routine use in the laboratory. My colleague, Dennis Slone, and I spent many months working with M. Grinbergs, our technician, to try to identify the critical variables so that this conceptually excellent assay would have sufficient reliability and accuracy for multiple uses in the laboratory.

"After setting up what could have been the 100th in a series of variations, the final incubation period was both started and completed (as usual) within one morning. It happened to be in this particular instance on a Friday. The centrifugation step to separate the putative bound from the putative free insulin was accomplished, the supernatant was separated from the precipitate and the tubes were placed into an automatic changing

gamma counter. This trial assay was rapidly counted and calculated and Slone, Grinbergs, and I saw once again that the assay had failed. By this time it was late in the afternoon of that Friday so rather dejectedly we left the assay tubes in the tube holder of the gamma counter and went our unhappy ways for the week-end.

"On Monday morning, we commenced preparations for another trial radioimmunoassay. As we were removing the tubes from the unsuccessful assay of the previous Friday, to our amazement we noticed that a spontaneous precipitate had formed in the tubes containing the supernatant which we had never previously detected because after each previous trial assay, we inevitably removed and discarded the tubes from the gamma counter. We immediately re-centrifuged the supernatant tubes and counted this 'second' precipitate. When the radioactive counts of the second precipitate were added to the first precipitate and the counts of the supernatant appropriately adjusted, our calculations quickly revealed that the assay had performed exceptionally well. All of the serum samples assayed at multiple dilutions and all of the recoveries now fell into line. We now realized that the most critical variable for the double antibody radioimmunoassay had been identified and within the next few months we did an extended series of studies with both serum and plasma using a wide range of the second incubation times and were able to develop the data that led to the manuscript.

"Prior to publication, I was able to present this at the Fifth Congress of the International Diabetes Federation, held in Toronto, Ontario, Canada on July 1, 1964.

"I suspect that since the study showed in detail how to perform accurate radioimmunoassays using the double antibody technique, many investigators around the world in diabetes and endocrinology rapidly adopted the assay for their particular studies.

"I estimate that over 100 individuals from other laboratories visited us to learn the technique from Grinbergs."

1. **Morgan C R & Lazarow A.** Immunoassay of insulin: two antibody system. Plasma insulin levels of normal, sub-diabetic and diabetic rats. *Diabetes* 12:115-22, 1963.