

This Week's Citation Classic

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Nieschlag E & Loriaux D L. Radioimmunoassay for plasma testosterone. *Z. Klin. Chem. Klin. Biochem.* 10:164-8, 1972, [Reproduction Research Branch, National Institute of Child Health and Human Development, NIH, Bethesda, MD]

The paper describes one of the first radioimmunoassays for testosterone, including the generation of the antiserum with a novel immunization technique. The method is based on the isolation of testosterone by thinlayer chromatography and is applied to plasma samples. [The SC[®] indicates that this paper has been cited over 185 times since 1972.]

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"When I joined M.B. Lipsett at the Reproduction Research Branch of the National Institutes of Health in early-1971, we needed assays for steroids that would allow serial analysis in small volumes of serum. At that time, the first radioimmunoassay for a steroid had just been 'invented' and D.L. Loriaux and I set out to apply this principle to the determination of estrogens and androgens, respectively. When immunizing rabbits with the testosterone conjugate we applied a new immunization technique which was simultaneously applied by J.L. Vaitukaitis to raise antibodies against the hCG subunits. This extremely useful method became another Citation Classic in 1980.¹

"When the radioimmunoassay for testosterone was established, I wanted to publish it. Since, however, one radioimmunoassay for testosterone had already been published² (to date there are over 50 published modifications!), it was decided that the manuscript should be submitted 'only' to a

German journal and not, as I had hoped, to one of the larger American journals. The paper received some unfriendly comments from one of the reviewers. This reviewer, however, did not hesitate to be one of the first to ask for an antiserum sample, as I learned several years later.

"Soon after publication many people requested the testosterone antiserum and to date my records show that 140 laboratories from all over the world have received samples, several of them repeatedly. A sample of testosterone antiserum has become a well-appreciated gift when visiting other laboratories. While others tried to reap financial reward from their steroid antisera, we gave the samples away free of charge. The antiserum was used, and in many instances also our method, obviously with success. The successful application of this method to many physiological studies explains the frequent citation of the paper.

"After my return to the Federal Republic of Germany in 1972, the method remained a standard method for some time at the Reproduction Research Branch. In the Federal Republic of Germany I applied it to several investigations on the physiology and pathophysiology of testicular function.³ The method enabled us to investigate, among other topics, the declining testicular function in senescence and to find a correlation between circulating testosterone levels and the male vocal register. Having immunized a number of animals for the production of antisera for assay purposes, we became interested in the biological effects of the testosterone antibodies in the immunized animals. In my opinion, studies in this area arising as a spin-off from the testosterone radioimmunoassay became more important than the assay itself."⁴

1. **Vaitukaitis J L, Robbins J B, Nieschlag E & Ross G T.** A method for producing specific antisera with small doses of immunogen. *J. Clin. Endocrinol. Metab.* 33:988-91, 1971. [Citation Classic. *Current Contents/Life Sciences* 23(33):12, 18 August 1980.]
2. **Furuyama S, Mayes D M & Nugent C A.** A radioimmunoassay for plasma testosterone. *Steroids* 16:415-22, 1970.
3. **Nieschlag E & Wickings E J.** The role of testosterone in the evaluation of testicular functions. (Abraham G E, ed.) *Application of radioassay systems in clinical endocrinology.* New York: Dekker, 1981. p. 169-96.
4. Biological effects of antibodies to gonadal steroids. (Munson P L, Glover J, Diczfalusy E & Olson R E, eds.) *Vitamins and hormones: advances in research and applications.* New York: Academic Press, 1978. Vol. 36. p. 165-202.