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Mancini G, Carbonara A O & Heremans J F. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 2:235-54, 1965. [Research Department of Internal Pathology, Cliniques Universitaires St. Pierre, Louvain, Belgium]

This paper describes a new, very simple, sensitive, and accurate immunochemical technique—radial immunodiffusion (RID)—for the quantitative evaluation of antigens in body fluids. This technique has since been employed on a wide scale, both in research laboratories and even more so in clinical practice. [The SC[®] indicates that this paper has been cited in more than 10,240 publications, making it the most-cited article published in this journal]

Refining the Angelotron

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I entered the Research Department of Internal Pathology (headed by J.F. Heremans), Cliniques Universitaires St. Pierre, Louvain, Belgium, at the age of 28, in May 1962, after obtaining my degree in medicine and surgery and a post-graduate diploma in hematology from the University of Rome. My lab experience at that time had been confined to electrophoretic and immunoelectrophoretic analyses for diagnostics and clinical purposes. I quickly settled down in this new environment, because I had already spent a month in this laboratory in August 1961 and knew Heremans and his colleagues P. Masson, J.J. Picard, J. Rodhain, and J.P. Vaerman. In addition, another Italian, Angelo Carbonara, with whom I had worked in Rome and who subsequently became my husband, had been there for nearly a year.

One of the lab's research programs was directed toward the immunological evaluation of plasma proteins. At that time, the only technique available was the simple tube immunodiffusion test introduced by J. Oudin in 1949.¹ This was widely used by Heremans and other lab researchers. However, it had significant limitations, primarily due to technical difficulties, poor sensitivity, and, above all, many artifacts that hampered the interpretation of the results.

It was Carbonara who first contemplated the possibility of using, radial immunodiffusion

(RIO), then exploited solely at a semiquantitative level. When I arrived at Louvain, in fact, it was jokingly referred to as the "Angelotron." However, it lacked the mathematical relations between the individual antigen-antibody precipitate diffusion area and the antigen concentration for use in quantitative assessment. The task of discovering these relations was placed in my willing hands. After much patient effort and many attempts, and with the enthusiastic assistance of Heremans, I was able to show that, when an unknown amount of antigen is allowed to diffuse radially from a well in a uniformly thin layer of antibody-containing agar for a sufficient time, the final area reached by the precipitate is directly proportional to the amount of antigen employed, and inversely proportional to the antibody concentration.

RIO was publicly presented for the first time at the 10th Protides and Biological Fluids Meeting held in Bruges in 1963.² It also formed the subject of my degree dissertation ("Science Medicales") the following year.

It was quickly employed on a wide scale, and it was not long before plates containing antisera against various plasma proteins were made available by several companies. The method was primarily used in a clinical context for disorders associated with quantitative changes in serum proteins. Until the advent of nephelometry, indeed, it was unrivaled. The method also has been adapted to the qualitative analysis of antigen relationships among different substances.^{3,4}

We three authors agreed not to patent the method and never regretted our decision. Nor has my discovery ever brought any form of reward or honors. I came back to Turin in 1965. After five years at the university, I took up hospital work and am currently head physician at the Sant'Anna Gynaecological and Obstetrics Hospital analysis laboratory in Turin. My husband is professor of medical genetics at Turin University. We have two daughters aged 24 and 22, one reading medicine, the other architecture. Looking back on RID over this span of 30 years, I feel my greatest satisfaction was that of working with Heremans, whom I greatly esteemed and admired, and of collaborating with so many friends at Louvain, with whom I am still in touch. A single note of sadness must form the epilogue to this brief account: the death of Heremans—the loss of a maestro and a friend.

1. Oudin J. La diffusion d'un antigene dans une colonne de gel contenant les anticorps precipitants homologues: etude quantitative de trois principales variables. *C. R. Acad. Sci.* 228:1890-2, 1949. (Cited 115 times.)
2. Mancini G, Vaerman J P, Carbonara A O & Heremans J F. A single radial diffusion method for the immunological quantitation of proteins. (Peeters H, ed.) *Protides of biological fluids*. Amsterdam. The Netherlands: Elsevier. 1964. p. 370-3. (Cited 535 times.)
3. Mancini G, Nash D R & Heremans J F. Further studies on single radial immunodiffusion. III. Qualitative analysis of related and unrelated antigens. *Immunochemistry* 7:261-4, 1970.
4. Heremans J F. Antigen titration by simple radial immunodiffusion in plates. (Curtis W A & Merrill W C, eds.) *Methods in immunology and immunochemistry*. New York: Academic Press. 1971.

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