

Laurell C B. Antigen-antibody crossed electrophoresis.

Anal. Biochem. 10:358-61, 1965.

[Dept. Clinical Chemistry, Malmö General Hosp., University of Lund, Sweden]

Antigen-antibody crossed electrophoresis and crossed immunoelectrophoresis are alternative collective terms for semiquantitative techniques for analyzing charge heterogeneity and type of immunologic precipitation reaction of macromolecules carrying a common antigenic determinant. [The SCI® indicates that this paper has been cited in over 1,290 publications since 1965.]

Carl-Bertil Laurell
Department of Clinical Chemistry
University of Lund
Malmö General Hospital
S-214 01 Malmö
Sweden

February 5, 1985

The "antigen-antibody electrophoresis" technique was developed at our department.

An agarose gel electrophoresis run on cooled glass plates was introduced at our laboratory in the early 1960s for separating native plasma proteins. However, when Grabar and Williams's¹ immunodiffusion principle was used to identify specific proteins, the narrow zone obtained for any given protein was blurred as an extended bow. Having studied the kinetics of immunoprecipitation in free solution, I decided to test whether the slow diffusion step in immunoelectrophoresis could be exchanged for a faster immunoprecipitation technique by forcing the proteins separated by electrophoresis through an agarose gel containing an admixture of antibodies. Since my primary interest was α_1 -antitrypsin's electrophoretic heterogeneity and genetic variants, the initial precipitation experiments were run with rabbit antisera to human α_1 -antitrypsin. After agarose gel electrophoresis of plasma, a gel strip was cut out along the migration path of the proteins and transferred to the surface of an antibody-containing agarose. An electric field was applied perpendicular to the earlier migration path of the proteins. Narrow precipitation peaks were obtained within a few hours instead of the extended precipitation bows a day after immunoelectrophoresis.

An experiment with goat antiserum raised to total human proteins was tested to investigate the general applicability of the technique. An elegant pattern of precipitation peaks was obtained and presented in this brief report in *Analytical Biochemistry*, but few if any researchers reading the report realized the potency of the technique to gauge the charge heterogeneity of native proteins. An exception was Tristram Freeman from Mill Hill, a frequent visitor at our laboratory. He simplified the technical procedure at the cost of protein resolution, though this did not concern him as his purpose was to be able to quantify several plasma proteins simultaneously by measuring the peak heights of the various immunoprecipitates.

Niels Harboe, director of the protein research laboratory at Copenhagen University, grasped the technique's potential in immunochemical work and supported the work of a group of able and enthusiastic researchers in modifying and applying the technology. Their experience was collected in a series of papers in the second supplement of the *Scandinavian Journal of Immunology*.² At our own unit, emphasis has been on the technique's high resolving capacity with a view to obtaining characteristic immunoprecipitation patterns for use in establishing degrees of antigenic identity. This was achieved when the gel strips were inserted into the antibody-containing gels instead of being placed on their surfaces. The joint experience of electro-immunochemical techniques by our staff was summarized in 1972³ and that of the Copenhagen group in 1983.⁴

Freeman termed his modification of antigen-antibody crossed electrophoresis *Laurell electrophoresis*, later altered by his pupils to *Freeman electrophoresis*. Today the various two-dimensional variants are collectively referred to as *crossed immuno-electrophoresis*. The first step of protein separation may vary (agarose, starch gel, polyacrylamide gel electrophoresis, or isoelectric focusing), and is followed by perpendicularly crossed electrophoresis through an antibody-containing agarose (immuno). In assessing degrees of electrophoretic heterogeneity or of antigenic identity, these methodological variants are powerful and widely used tools, which accounts for the frequency of references to the method by either of its collective names.

1. Grabar P & Williams C A. Méthode permettant l'étude conjuguée des propriétés électrophorétiques et immunochimiques d'un mélange de protéines. Application au sérum sanguin. *Biochim. Biophys. Acta* 10:193-4, 1953. (Cited 1,700 times since 1955).
2. Axelsen N H, ed. Quantitative immuno-electrophoresis: new developments and applications. (Whole issue.) *Scand. J. Immunol.* 4(Suppl. 2), 1975, 230 p.
3. Laurell C B, ed. Electrophoretic and electro-immunochemical analysis of proteins. (Whole issue.) *Scand. J. Clin. Lab. Invest.* 29(Suppl. 124), 1972, 136 p. (Cited 1,555 times.)
4. Axelsen N H, ed. Handbook of immunoprecipitation-in-gel techniques. (Whole issue.) *Scand. J. Immunol.* 17(Suppl. 10), 1983, 383 p.