
Preface

Amazingly for a procedure so fundamental to present-day biomedical research as well as medical diagnostics, development of the western blot occurred within the memory of many individuals who continue to work in these fields. That is, conversely, there are many investigators and clinicians presently working who recall the days prior to the western blot. The personal stories of how and why the technique was developed are given in this volume by the people intimately involved with this development. These stories date from only roughly 30 years ago. Since then, a very large number of variations on the theme of gel separation of biologic products by different parameters such as size or charge, followed by transfer to solid support and identification, have been developed. The present compilation brings together a large number of these techniques, some of which are adaptations of the original technique in order to solve a problem, whereas others are far flung and vastly different from how the original techniques were envisioned. The goal of our work is to not only compile the vast array of techniques based on western blot, but also give practical methods. We suspect that almost everyone involved in the enterprise has tried to bring a new technique to their laboratory by reproducing methods found in traditional publications. Doing this is commonly fraught with difficulty and may take weeks to accomplish, or may be abandoned as impossible. We hope that investigators will be able to open this volume and rapidly begin to use a technique new to them and their laboratories because the chapters give detailed practical methods, tips, and alternatives. If you are able to open a chapter, and conveniently and quickly perform a new procedure in your laboratory, then we will have accomplished our goals in editing this work.

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Chapter 2

Western Blotting: Remembrance of Past Things

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Summary

Western blotting sprung from the need to develop a sensitive visual assay for the antigen specificity of monoclonal antibodies. The technique employed SDS-PAGE of protein antigens, electrophoretic replica transfer of gel-resolved proteins to unmodified nitrocellulose sheets, probing the immobilized antigens with hybridomas, and detection of antibody–antigen complexes with radiolabeled staphylococcal protein A and autoradiography. The simplicity and relevance of the method has led to its expansive application as an immunodiagnostic and a ubiquitous research tool in biology and medicine.

Key words: Western blotting, SDS-PAGE, Electroblothing, Unmodified nitrocellulose, Immobilized replica, Antigen specificity, Antibody–antigen complex, Protein A

Paraphrasing Plato, Jonathan Swift once famously observed that “Necessity is the Mother of Invention” (1). Such necessity was the antecedent of western blotting. The fact that similar techniques arose within the same time frame indicates the temporal pressure of an unfilled demand in biology and medicine – a common exigency to provide a tool by which to visualize specific antigens.

The requirement that impelled the development of western blotting (2) in my laboratory came to light in 1977, when I moved to Robert Nowinski’s RNA tumor virus group at the Fred Hutchinson Cancer Research Center. This was just at the time when monoclonal antibodies were first described by Köhler and Milstein (3), and Bob’s group was developing monoclonal reagents as probes to assess the structural and immunologic nature of retrovirus proteins (4). It quickly became clear that there was no simple, objectively visual way to easily screen the vast numbers of

generated clones for their specificity toward individual structural polypeptides comprising the retrovirus envelope and core.

Although the main focus of my work at the time was in other areas of retroviral research, I had a methodological background in electrophoretic antigen assessment; therefore, I agreed to undertake the effort in the Nowinski group to develop new and streamlined techniques to facilitate screening of the hybridomas for antigen specificity. Having been trained as a postdoc in Tom August's lab at Albert Einstein College of Medicine in radioimmunoassays, immunoprecipitation, and SDS-polyacrylamide gel electrophoresis (SDS-PAGE), I attempted to conceive of ways in which these methods might be combined. RIAs had great sensitivity, but lacked the ability to give a simple picture of specificity, especially in complex protein mixtures. Conversely, immunoprecipitation required radiolabeling of diverse antigen species and, while it provided reasonable sensitivity and definition of specificity when linked to SDS-PAGE and autoradiography, it was plagued by significant background that led to substantial uncertainty and was not easily adaptable to high-throughput screening.

Launching into this project, essentially on my own and without benefit of knowledge of others who might be engaged in similar work, I attempted a wide array of techniques, hoping that I would stumble upon something useful or, at least, something that might light the pathway to proceed further. Here, I was trying through trial-and-error to fulfill another Swifitian dictum: "Discovery consists of seeing what everybody has seen and thinking what nobody else has thought" (1). In retrospect, some of the things I tried verged on the laughable. Nevertheless, the early work furnished me with the recognition that purified, radiolabeled (in this case, radioiodinated) staphylococcal protein A (5) provided a more functionally stable and "universal" imaging agent for detection of antigen-antibody complexes than did "second antibody" reagents.

As incongruous as it might seem in the hindsight of nearly 30 years, I struggled with how to apply the monoclonal antibodies (as well as monospecific antisera) to gel-separated antigens. The "Eureka" moment occurred while I was concomitantly performing other experiments that employed "Northern" blots (6), an effulgent clarity of vision that an immobilized "replica" of the PAGE-resolved proteins was to be an intrinsic element. Initially, I attempted passive transfer by placing gels in direct contact with derivatized, and later unmodified, nitrocellulose sheets. After overcoming problems associated with nonspecific binding of immunoglobulin and protein A reagents to the nitrocellulose by the use of a blocking agent (I employed immunoglobulin-depleted, purified bovine serum albumin), it became apparent that capillary transfer was slow, inefficient, and resulted in unacceptable diffusional band-spreading of the gel-resolved antigens.

A second Archimedean moment occurred at this point, when I came across an old electrophoretic gel destainer that I had not used for years. Perhaps, I reasoned, if I could work fast enough or keep temperatures low enough to minimize band diffusion *within* the parent gel, and find electrophoretic conditions and nitrocellulose pore size to prevent driving the proteins out of the gel and *through* the paper, I might be able to make better “replicas” of the gel-resolved antigens.

It only took about a week from this point to work out the “final” parameters of the basic electroblotting technique, and another few weeks to work on adaptations that could increase resolution and sensitivity in complex mixtures (e.g., cell culture, blood, tissue, and other clinical samples) using isotachopheresis in a first dimension, then applying such cylindrical gels to the SDS-PAGE slab gels. During this period, a manuscript was prepared and a discussion with Bob Nowinski ensued wherein the name “western blotting” was conceived. It was just at this time that the publication of Towbin et al. (7) appeared. Although the basic technique described by these investigators was similar, I believed that many of the simplifying and “universalizing” aspects of western blotting (e.g., unmodified nitrocellulose, radiolabeled protein A detection, 2-D separations, etc.) were sufficiently important to warrant submission of my manuscript. I also became aware at this time of the publication by Renart et al. (8); however, the technique described in their paper employed conditions with which I had experimented (e.g., derivatized paper, passive capillary transfer, second antibody, etc.) and found wanting from the perspectives of simplicity, ease of use, resolution, sensitivity, and specificity.

The manuscript was submitted to *Analytical Biochemistry* and was rejected without, it seemed, any recourse to resubmission. It was interesting to note that the rejection appeared to me to be based not on any technical criticisms or its ostensible similarity to the methods of Towbin et al. (7), but rather on the reviewers’ sentiment of the pedestrian nature of the contribution and, particularly, to the flippant and frivolous whimsy in the name “western blotting.”

As previously documented (9), preprints of the rejected manuscript had been sent to colleagues, who subsequently provided them to others, and they to others until, eventually (even in this preelectronic era of written communications), it seemed as though this unpublished article had received wider distribution than many published ones. I only became aware of this subsequent to my move to the Salk Institute at the end of 1979. It was there that I was tracked down and spent a good part of every work day fielding telephonic questions about the technique and providing readable copies of the preprint – the original I had sent to a few colleagues had undergone many cycles of photocopy replication as it wended its way from lab to lab, the later generations being difficult to read. After about a half year

of operating this private “journal club,” I called the editor-in-chief of *Analytical Biochemistry*; he agreed that the situation was untenable, that the general immunoblotting technique (as well as the name “western blotting”) was becoming widely accepted, and that the initial rejection of my manuscript was probably unfortunate. Therefore, I resubmitted the paper (with only very minor changes); it was accepted immediately, and finally published a few months later (2).

For those who have felt the sting of journal rejection, it is worth noting that this paper has entered a small pantheon of the most highly cited scientific articles, all of which were initially rejected for publication (10). Humility is an oft-reinforced virtue in science; it is humbling to realize that this little paper on western blotting far transcended the sum of journal citations for all of my other published research efforts. Nevertheless, it is a source of immense satisfaction to have made – along with Towbin et al. (7) – a lasting contribution to the methodological armamentarium of biological and medical scientists.

To complete the analogy hinted in the title of this review, I wish to thank the editors of this volume for providing me, like the proffered “madeleine” in Proust’s *À la recherche du temps perdu* (11), the occasion for this reminiscence.

References

1. Swift, J. (1726) *Travels into Several Remote Nations of the World, in Four Parts. By Lemuel Gulliver, First a Surgeon, and then a Captain of several Ships*. Benjamin Motte, London.
2. Burnette, W.N. (1981) “Western blotting”: electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* 112, 195–203.
3. Köhler, G. and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256, 495–497.
4. Nowinski, R.C., Stone, M.R., Tam, M., Lostrom, M.E., Burnette, W.N., et al. (1980) Mapping of viral proteins with monoclonal antibodies: analysis of the envelope proteins of murine leukemia viruses, in *Monoclonal Antibodies: Hybridomas: A New Dimension in Biological Analyses* (Kennett, R.H., McKearn, T.J., and Bechtol, K.B., eds.), Plenum, New York, pp. 295–316.
5. Langone, J.J. (1978) [¹²⁵I]protein A: a tracer for general use in immunoassay. *J Immunol Methods* 24, 269–285.
6. Alwine, J.C., Kemp, D.J., and Stark, G.R. (1977) Method for detection of specific RNAs in agarose gels by transfer to diazobenzoyloxymethyl-paper and hybridization with DNA probes. *Proc Natl Acad Sci USA* 74, 5350–5354.
7. Towbin, H., Staehelin, T., and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76, 4350–4354.
8. Renart J., Reiser, J., and Stark, G.R. (1979) Transfer of proteins from gels to diazobenzoyloxymethyl-paper and detection with antisera: a method for studying antibody specificity and antigen structure. *Proc Natl Acad Sci USA* 76, 3116–3120.
9. Burnette, W.N. (1991) Western blotting (Citation Classic). *Curr Contents* 34, 8.
10. Campanario, J.M. (1996) Have referees rejected some of the most-cited articles of all times? *J Am Soc Inf Sci* 47, 302–310.
11. Proust, M. (1913) *À la recherche du temps perdu* (vol. I). Bernard Grasset, Paris.