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40 years Western blotting: a scientific birthday toast

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Author-blinded main text

Abstract

The Western blot technique was first published 40 years ago. Since then, it has been mentioned in the titles, abstracts, and keywords of more than 400,000 PubMed-listed publications. This anniversary represents an opportunity to look back to its origins, to address the question as to whether the technique is outdated and heading toward being replaced, and to point out current improvements. Publications within the last ten years indicate Western blotting may be the most used protein-analytical technique. Although no decline in its usage is visible, the method has been moving out of the spotlight.

Highlights

- The Western blot technique was first published 40 years ago
- Western blotting may still be the most used protein-analytical technique
- No decline in its usage is visible, although it moved out of the spotlight
- Improvements of its reproducibility and precision are ongoing.

Keywords: Western blotting, Western blot, Immunoblotting, Immunoblot, protein analytical methods, mass spectrometry

Introduction

40 years of Western blotting – Happy birthday! From an academic perspective, an age of 40 years is often associated with a phase of high performance, the first permanent position – or the end of a career. What is the "career" of Western blotting? Has it established a permanent position in the biomedical laboratories of the world, or has it retired and been left behind in favor of more modern methods like targeted mass spectrometry [1]? These questions will be addressed in this birthday toast article.

The Past: Three Parents and Multiple Applications

Western blotting is an analytical method by which separated proteins are transferred to a membrane enabling an estimation of the presence, relative quantity, molecular weight, and modifications such as degradation or posttranslational modifications of one or more targeted proteins.

Quite progressive for its time, around the 1980s, Western blotting has more than two parents. Three scientific publications are commonly mentioned when discussing the origin of Western blotting. The very first – the actual birth of the technique - was published by Renart and colleagues from the group of George Stark on July 1, 1979 in PNAS [2]. This group had already invented the RNA blotting technique named "Northern blot" two years prior [3], the first of a series of puns used in blotting names referencing Edwin Southern's invention of the DNA blotting technique called "Southern blot" [4]. This first publication of Western blotting from 1979 used capillary force for the transfer, polyacrylamide/agarose gel mix as the separation matrix, diazobenzyloxymethyl paper as the target membrane, and ¹²⁵I-labeled Protein A for detection [2].

Only two months later, unaware of each other's work [5], Harry Towbin and colleagues published a similar approach in the same journal [6]. However, instead of capillary transfer, they used electrophoretic forces to transfer the proteins. Furthermore, they applied SDS-

and Urea-PAGE as a separation gel matrix, nitrocellulose membranes as their transfer target, and primary as well as secondary antibodies for protein detection.

Almost two years on, W. Neal Burnette published the renowned nickname for the baby for the first time – and continuing the pun – “Western blotting” [7]. Both Burnette and Stark’s labs were located on the west coast of the USA at that time, hence another reason for calling the method “Western” blots [5]. Burnette’s variant was quite close to that of Towbin and colleagues in using SDS-PAGE for protein separation, electrophoretic transfer and nitrocellulose as the immobilization target, but it used radiolabeled Protein A for detection. Interestingly, Burnette’s article had been prepared at the same time as the other two articles appeared. It was rejected, however, by *Analytical Biochemistry*; the same journal that changed its mind in 1981 [8].

Compellingly, the citation numbers differ considerably among the three publications. As it happens, the very first article has been cited the least frequently of the three, with 978 citations. The second from Towbin et al. has 58,462 citations, substantially more than the other two, most likely because the technique published in this article is still closest to today’s most common version of Western blotting which includes the unchanged transfer buffer (“Towbin buffer”). Burnette’s article received 8,660 citations (citation numbers from scholar google, October 18, 2019).

In its first 15 years, the Western blotting technique developed into an important diagnostic method. For example, as announced in the *New England Journal of Medicine* in 1986, the Creutzfeldt-Jakob disease could be diagnosed via Western blot-based detection of marker proteins in human brain tissue [9]. Western blot testing was also applied for serological diagnosis of human immunodeficiency virus infection [10], but later criticized as not well-standardized and not reproducible enough [11].

Since the 1990s, Western blotting was involved in fraud scandals and rejections, which damaged its reputation and raised general concerns over the method and its ability to semi-

quantify protein levels. In the light of dozens of paper rejections and even some ended careers due to misuse of Western blots, Marcus and Oransky, in their publication entitled *Can We Trust Western Blots?*, attempted to give an answer to the very question in the title with the subheading *Not without more transparency and scrutiny*. [12] Their project “Retraction Watch” follows and manages all article retractions and lists more than 200 blog entries containing the search term “Western blot”¹ (*nota bene*: some of them discuss the same case). Bik et al. screened 20,621 research papers published from 1995 to 2014 containing the search term “Western blot” for inappropriate duplications of photographic images. Almost 4% (782 papers) had at least one figure showing inappropriate duplications [13]. Although their publication does not specify whether this duplication concerns the Western blot image, an educated guess by the first author estimated a proportion of around half of the suspicious cases were Western blot images (personal communication by Elisabeth M. Bik). Aside from misuse, Western blotting has suffered from surprisingly high numbers of commercial antibodies of doubtful to insufficient quality [14–16], which – probably not alone – has caused severe reproducibility issues [17]. Nevertheless, it has to be noted that Western blotting is at the same time a valuable means for antibody validation [18,19].

The Presence: Steadily Used without Setback but Leaving the Spotlight

Despite the distrust and criticism, accurately quantifying relative protein levels is possible, given that antibodies are working. In everyday lab work, many researchers may have experienced themselves that issues with the technique are rather associated with incompetent and/or conservative my-lab-has-always-done-it-this-way researchers than with flaws inherent to the method. However, successful, reproducible, and [semi-]quantitative application is possible by following appropriate experimental procedures and by determining

¹ <https://retractionwatch.com>, Oct. 16th, 2019

the linear and quantitative dynamic range for each target protein under the given experimental conditions [20,21].

Where is the technique today, 40 years after its birth? Is it outdated, replaced by targeted mass spectrometry (as suggested at least for quantifying proteins in complex samples [1]) and starting its midlife crisis? To answer these questions, one may check how much it is still used in labs around the world. To do this in a relatively scientific way, one may check the numbers of citations and compare them with those of mass spectrometry and other techniques. Given that the number of publications is generally continuously increasing, one should normalize the numbers with the paper number in the protein-related research field, for instance. For 2018 for example, PubMed lists 194,262 protein-related publications, of which 16,196 (8.3%) mention Western blots. The proportion of protein-related publications applying Western blots has stabilized at a value of around 8-9% over the last 30 years (Fig. 1A; details regarding search terms in the subtext of Fig. 1; absolute numbers and percentage values for each year and search term in Suppl. Tab. 1). This applies of course only under the assumption that mentioning and performing Western blotting are roughly equivalent. Note that these numbers may be underestimated since the number of publications applying Western blots without mentioning it as a "Text Word" [22] might be much higher than the reverse. Doing the same quantification for mass spectrometry results in a relatively stable value of 4% over the last 10 years (Fig. 1B). This suggests that (1) Western blots are not only steadily used by the protein community but that (2) they are applied even roughly two times more often than mass spectrometry, probably due to lower costs and complexity. Furthermore, compared to other analytical techniques commonly used in protein science, such as ELISA (3%), IHC (4-6%), and microscopy (4-5.5%), Western blotting appears to be the most used technique in protein-related publications within the last ten years (Fig. 1A-E).

Nevertheless, even in a birthday toast article, a serious scientist must be neutral enough to detect downward developments. When totaling the number of publications mentioning

Western blotting and other techniques in their *title* (normalized in the same way as before), the situation turns. In detail, the proportion of protein-related papers mentioning Western blots in their title reached its peak in 1989 with 3,4 permille (‰, i.e., per thousand; absolute numbers: 156 out of 45,630) but stabilized at values of below 0.5‰ during the last 10 years. Mass spectrometry, in contrast, has been reaching a stable high proportion of around 5-6‰ in the last 10 years. Hence, in this title-centric view, mass spectrometry even outperforms other techniques such as ELISA (1‰), IHC (0.5-1‰), and microscopy (2‰). These data hint that Western blotting, albeit steadily used without any setback, is moving out of the spotlight in publications, in contrast to mass spectrometry.

Nonetheless, with marked technical improvements, such as introducing microfluid designs and single-cell Western blots, the technique still features in the titles of high-ranked journals of even the current decade [23,24].

Future: Miniaturization and Other Improvements

These instances of Western blotting remaining in the spotlight of publications may indicate future developments in Western blotting. For example, current work is being done to improve the technique in terms of faster application and miniaturization by using capillary electrophoresis or isoelectric focusing instead of PAGE [25,26]. Notably, the actual “blotting” part is adapted in these cases, as the protein does not have to be transferred, but simply immobilized in a capillary. It could lead to semantic discussions regarding what still falls into the category of Western *blotting*. Further improvements concern the internal loading control which is heading from housekeeping proteins to total protein staining [27–30] and progress towards the technique’s usage in immunological diagnostics [31–34]. Improvements in different areas of Western blotting are summarized in Mishra et al [35]. Regarding the reproducibility issue caused by misuse, qualitatively poor antibodies, and a lack of relevant method information in publications, Gilda and colleagues have proposed a Western blotting

minimal reporting standard, which should be applied for all publications using Western blotting [17]. Overall, the future of Western blotting not only depends on technical progress, but also on the integrity and competence of lab staff, scientists, and antibody producers.

Conclusion

Forty years after its first publication, Western blotting is not outdated at all. Based on calculations in this article, I estimate that among published protein-related projects, Western blotting has been applied at least two times more often than other techniques, such as MS, ELISA, IHC, or microscopy within the last 10 years. However, as indicated by the decreasing number of mentions in article titles, Western blotting may have moved out of the spotlight in research projects after its heydays in the late 1980s and early 1990s. Although the principal processes of Western blotting have not much changed since its conception, innovative approaches have improved the method with higher reproducibility, faster applications, and minimized sample consumption.

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Figure:

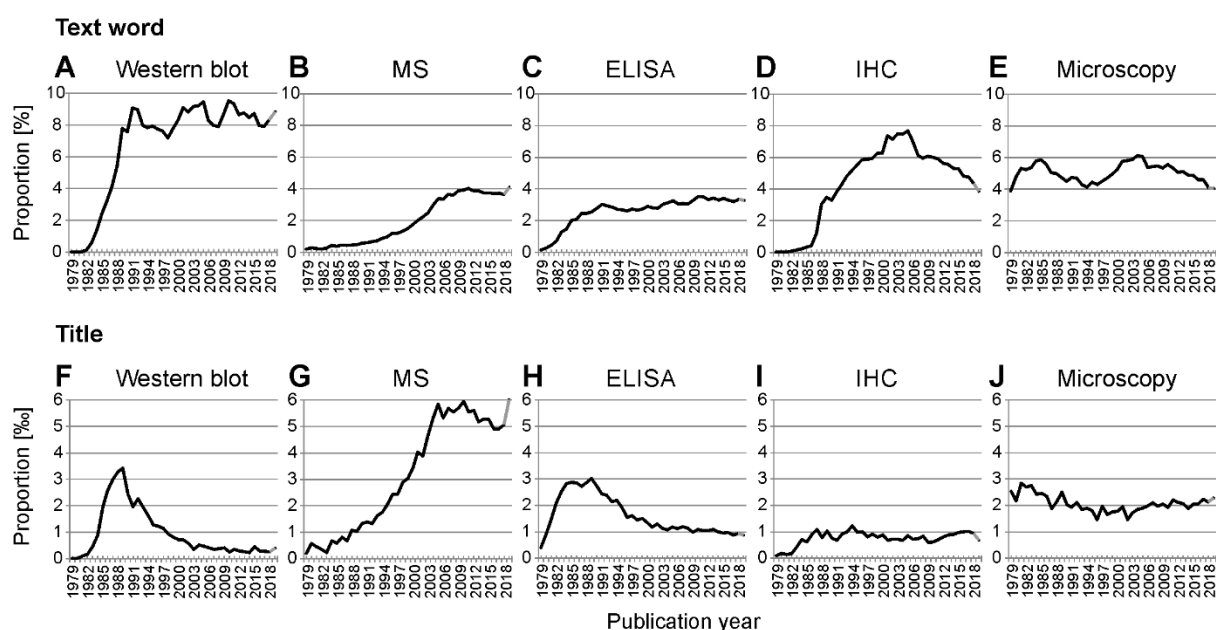


Figure 1: Proportions of application of five different techniques in relation to all protein-related publications estimated by the number of PubMed mentions in the *Text words* or *Title*. The relative frequency of usage of Western blots, mass spectrometry (MS), enzyme-linked immunosorbent assay (ELISA), immunohistochemistry (IHC), and microscopy was estimated by counting the mentions of the techniques in the Text words² (A-E) or Title (F-J) in PubMed (option “Results per year” downloaded on August 20, 2019 as a csv file). In order to avoid counting non-protein-related articles mentioning mass spectrometry, such as mass spectrometry of small molecules, we searched for protein-related articles only $((protein*[Text\ Word]\ OR\ proteins*[Text\ Word]\ OR\ proteom*[Text\ Word])\ AND\ followed\ by$

² Text words comprise title, abstract, MeSH headings and subheadings, other terms field including keywords, chemical names of substances, secondary source identifier, personal name as subject [22]

the technique search terms). The following search terms for the five techniques were used: A, Western blot: (*Western blot*[Text Word] OR Immunoblot*[Text Word]*); B, MS: (*mass spectrometry[Text Word] OR mass spectrometric*[Text Word]*); C, ELISA: (*ELISA[Text Word] OR enzyme-linked immunosorbent assay[Text Word]*); D, IHC: (*IHC[Text Word] OR Immunohistochemistry[Text Word]*); E, microscopy: (*microscopy[Text Word]*). For the search restricted to the title (F-J), [*Text Word*] was replaced by [*Title*]. All numbers were set in relation to the total number of protein-related publications of the same year (A-E: percent, %; F-J: permille, ‰). Grey part of the graph: preliminary PubMed entries for 2019.

Graphical abstract:

