FIGHTING FOR THE FUTURE OF ANTIBODIES

Nathan Blow looks at how efforts to create guidelines and scoring systems could change the way you buy antibodies.

Credit: Asilomar Conference Center.

I had never been to the Asilomar Conference Center before. Located in California near Monterey, the name should be recognizable to most molecular biologists—Asilomar was the place where, in February 1975, a group of 140 molecular biologists, lawyers, and physicians came together to hammer out guidelines for recombinant DNA use in the lab.

It was September 2016, and I was driving my rental car from San Francisco to Asilomar to take part in a workshop organized by the Global Biological Standards Institute (GBSI). The goal was for attendees to focus on strategies to eradicate the problem of poorly validated antibodies in basic research. As I entered the grounds of the conference center, I was certain 2 days with the right people would be more than enough time to kick things off and, perhaps, even form a solid plan for the future. But as I started to speak with the scientists, journal editors, and antibody manufacturers in attendance, it quickly became clear the problem was significantly bigger than I had anticipated.

Antibody marketplace

At dinner that evening, I got my first glimpse of the challenges of using antibodies today. Among consumable reagents in life science, arguably none has seen wider and more varied experimental use than the antibody. In fact, it's been estimated that there are 500,000 to 1 million unique antibodies available for purchase today. More surprising might be the fact that by some estimates, the 300 companies selling antibodies offer nearly 3 million antibodies for purchase. This numerical discrepancy is the result of antibody licensing agreements between companies, making it possible to purchase the same antibody from two or

more different suppliers. The problem, according to my dinner companions, is how to know it's the same antibody.

Selling antibodies is a lucrative, multi-billion dollar business, which is the reason many cross-licensing agreements have been enacted. The extent to which the same antibodies are available from multiple vendors is not easy to determine, since in many cases information about their origin is not provided. Often, if an antibody from one company does not work for a researcher, they will buy a new antibody from another company. If that new antibody was part of a cross-licensing deal with the first company, the researcher would be trying the same experiment again with the same reagent without knowing it, wasting money and time, and potentially injecting a level of confusion into the data analysis.

This highlights an overarching problem in using antibodies today: lack of transparency. In many instances, researchers are given only limited information on the reagents that are being used in their experiments.

Most of the antibodies researchers know and use come straight from the well-worn pages of company catalogs, which generally provide basic information along with some validation data—likely the image of a western blot showing the antibody binding to a band of the proper molecular weight. Beyond this, researchers can search the scientific literature for additional information on a given antibody, but such information is not always available.

A single western blot image showing a band of the correct size means that an antibody binds a protein of the correct size, but within the area of that band, there could be hundreds to thousands of proteins co-migrating with the target protein. While good evidence, even the best

western blots cannot be considered definitive. During our 2-hour meal, I was starting to see the tip of the antibody validation iceberg.

This is not to say companies are at fault here. For manufacturers, the extent of antibody validation presents a unique challenge. “Antibody validation already is over 75% of production expenditure, so how do we balance the costs and the benefits effectively?” asks Will Olds, scientific officer at antibody manufacturer Proteintech. “If generating an antibody costs too much, then only the ‘hot’ ones with significant attention will be made, stifling exploratory science.” And then there is the question of what are the best experiments and conditions for validating an antibody—an antibody that works in an immunohistochemistry assay might not work for another type of assay, such as western blotting, and the reaction conditions of a given experiment can also influence the sensitivity and specificity of an antibody. This means that even when top-quality validation data are provided by the antibody producer, an antibody could still fail during an experiment if careful controls are not implemented.

Olds is not alone in these thoughts—I spoke to other antibody producers during the Asilomar workshop who also noted the difficult balancing act between being able to supply the large numbers of antibodies the life science community wants access to and the costs associated with those efforts.

Finding common ground

“What we really wanted to do at the Asilomar workshop was to raise awareness of the problem, define the issue and, ultimately, explain to the scientific community what we were going to do to address [the issue],” explains Len Freedman, president of GBSI. When I recently caught up with Freedman, it had been nearly a year since the Asilomar meeting, and he was hard at work trying to implement recommendations that emerged from the workshop.

Freedman is a scientist by training, but he left academia to head up GBSI in 2013. Speak with Freedman for even a short time, and you will quickly realize how driven he is when it comes to solving issues of reproducibility in scientific research. He has been advocating for years to create better standards and guidelines for biomedical reagents and data analysis. Freedman and GBSI have already tackled another reproducibility issue that has plagued the life sciences—cell line authentication. Studies showed re-

Will Olds, scientific officer at Proteintech, says coming together as a community and accepting mutual responsibility is the path to improving antibody validation practices.

searchers were, in some instances, using misidentified cell lines for their experiments, so GBSI set out to work with different groups to publicize the problem and help find a solution.

In comparison to antibody validation, cell line authentication can actually be viewed as a fairly simple problem. Cell lines need only a single assay for testing—short tandem repeat (STR) profiling—which costs less than \$200 to perform, and there are relatively few cell lines available to researchers. Compare that with the millions of antibodies that exist and the numerous different assays and varied conditions for which they are being used. Antibodies present an exponentially more complicated problem. And it’s not just the size of the problem—the economics have to be considered as well. A \$200 validation assay is one thing—validating an antibody using

multiple cells lines, transfection, or even knockdown experiments costs significantly more.

Even knowing the challenges, Freedman and GBSI have turned their sights towards antibody validation practices in recent years. Speaking with Freedman, I got the sense that he viewed the Asilomar workshop as a tipping point—an all-or-nothing attempt to change the way researchers and suppliers view the antibodies they use and manufacture on a daily basis.

Landscape leading to Asilomar

In the years before the Asilomar workshop, reports came out showing a growing trend of poor validation practices in multiple antibody-based studies. Several researchers actually suggest that the number of questionable antibodies being used in research today is much higher than what has been flagged in the literature. The problem, I was told, is that journals generally are not interested in publishing negative results, thus limiting the flow of information on problematic antibodies.

There have been attempts to define the scope of the antibody validation problem—in some instances with mixed reactions. In 2015, Freedman, along with Boston University economists Iain Cockburn and Timothy Simcoe, suggested in a controversial article in the journal *PLoS Biology* that \$28 billion was being spent every year on research that is not reproducible (1). Part of this lost money, Freedman and his colleagues said, is the result of poorly validated antibodies. While the article attracted media attention and interest, there were those who argued that the figure of \$28 billion was inflated, making the problem look larger than it actually was.

Putting the scope of the reproducibility problem aside for a moment, attendees at the Asilomar workshop agreed that the lack of standards and guidelines for how researchers should use antibodies was fueling the problem. While one supplier might provide information on how an antibody performed in an immunohistochemistry experiment, another might provide data from western blotting experiments. Sometimes experimental conditions critical to antibody performance are defined, and other times they are not. This can lead researchers to use an antibody validated for western blotting in a flow cytometry experiment, or vice versa, without realizing the validation data from the supplier has little relevance to the way they are actually using the antibody. The trouble is that validation for one type of assay does not mean the antibody works in all assays.

Among antibody users, journals, funding agencies, and manufacturers—the antibody world as it currently exists is truly the Wild West of science. There are no clear-cut rules on how to properly validate an antibody for a given assay or even what type of basic information should be provided. While access to research reagents is crucial, knowing what you are using in your experiments is even more important. This realization has led some to seek out a more formal definition of antibody validation.

Moving forward

Seven years ago, David Rimm and his colleagues started discussing standardizing antibody validation in a clever review article published in this journal (2). Rimm and his team examined the validation efforts used by dozen of antibody manufacturers and suppliers by searching through

catalogues and technical information. By implementing a simple 1–5 scoring system (5 being the best), they rated companies on how extensive and transparent their validation efforts were. Very few 5's were handed out.

Aside from publicizing the problem, Rimm's review also provided a flow chart for those looking to validate an antibody. This attempt towards standardizing antibody validation practices was comprehensive and intricate. The problem was that it was too comprehensive, actually deterring some from using it.

Following Rimm's article, others have tried to put forth guidelines, suggestions and recommendations for antibody validation. Most recently, a group led by Mathias Uhlen, professor of microbiology at KTH Royal Institute of Technology in Sweden, proposed five conceptual pillars for antibody validation in an article that came out just weeks before the Asilomar workshop (3).

In light of these efforts, a crucial question for those attending the Asilomar meeting was: What could be done to create a usable system for antibody validation that might be easily adopted?

Scoring solution

Attendee diversity was key when organizing the Asilomar meeting. According to Freedman, although GBSI invited key stakeholders from many different fields, they intentionally invited a large number of antibody manufacturers and providers. Freedman knew it would be crucial to get antibody producers tightly involved with any standards that might come out of the workshop.

During the second day, a general consensus began to form: The best solution might be to create a simple and transparent scoring system for antibodies. A single antibody score would make it possible for users to select reagents that had been validated with established criteria. It could also act as a simple guide for journals when it comes to implementing antibody validation policies. And a single scoring system would finally make validation criteria accessible to the scientific community. Debate on how such a scoring system might work followed, but at the end of the meeting, it was determined that there should be a concerted effort aimed at establishing an antibody scoring system.

An early antibody validation flowchart that was proposed by David Rimm and colleagues in 2010. Credit: D. Rimm.

Since the Asilomar workshop, GBSI has formed seven different working committees tasked with developing different scoring schemes for antibodies being used in seven of the most commonly used experimental applications. The outcomes of these efforts are slated to appear in the Fall of 2017—initially in the form of a beta test aimed at scoring a limited number of antibodies and evaluating the scheme before it is eventually rolled out for wider use. The big question is whether or not researchers and manufacturers will make use of the scoring system in the future.

“My personal wish is that there will be general adoption of the scoring system and that it becomes the standard when validating an antibody,” says Freedman. Still, he knows that buy-in from the scientific community can be difficult—his work with cell culture authentication and prior publications on antibody validation guidelines have shown him that. But the scoring system being suggested might have advantages over what has been previously tried—a single score is simple, and consumers often rely on scoring systems to make more informed purchasing decisions (as in the case of Consumer Reports), as long as the scoring is understandable.

One of those seven committees is focusing on how antibodies should be validated for use in western blot applications. Here, the members are working to develop a three-tiered scoring strategy that takes into account ex-

Len Freedman is president of the Global Biological Standards Institute (GBSI), the organization that hosted the 2016 Asilomar meeting focusing on antibody validation. Credit: GBSI.

periments used to assess an antibody’s specificity, sensitivity, and technical profile. Each antibody is scored based on the level of experimental rigor used in its validation—knockout experiments in cell lines followed by western blot analysis receive more points than antibody detection of purified protein run on a gel. Although each section is divided into multiple point values, in the end, a single number is generated that reflects the volume of antibody validation information for a particular antibody. With this, researchers can make more informed decisions about which antibodies they are using in their western blots or other experiments.

Overall, the current level of interaction between all stakeholders seems to be creating a positive environment for change. “We are ecstatic about the response of the entire community—from funders to manufacturers to journals and scientists—to address this problem. That willingness to cooperate and accept mutual responsibility are needed to remove this issue completely,” says Olds, who also attended the workshop at Asilomar.

Many articles have been written about the problem of antibody validation, but far fewer have focused on solutions. While no-one can tell what the future holds, I hope one day to be able to write a follow-up article on how the life science research community solved the problem of antibody validation. Asilomar worked for recombinant DNA—we shall see if it works for antibodies too.

References

1. **Freedman, L.P. et al.** 2015. The economics of reproducibility in preclinical research. *PLoS Biol.* 13:e1002165.
2. **Bordeaux J. et al.** 2010. Antibody validation. *BioTechniques.* 48:197-200.
3. **Uhlén, M. et al.** 2016. A proposal for validation of antibodies. *Nature Methods.* 13:823-7.

Written by Nathan Blow, Ph.D.

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