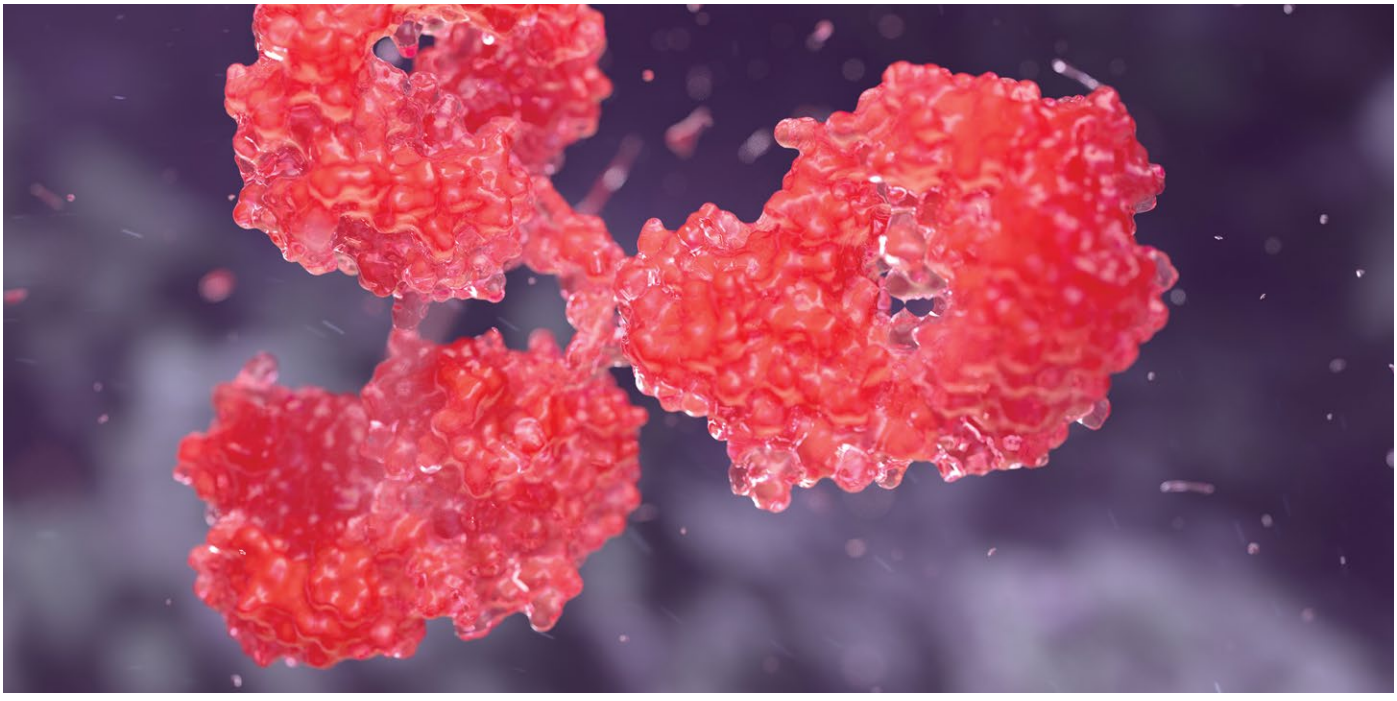

Agreeable antibodies: Antibody validation challenges and solutions

Commercially produced antibodies have become workhorses of basic research, but opaque supply chains, a lack of clear quality standards, and scientists' complacency have turned antibody-based experiments into potential disaster zones. Several strategies can help researchers navigate past these pitfalls. **By Alan Dove**

In the past few years, molecular biologists have come to a stunning realization: Some of their most critical reagents are so inconsistent that a large body of recent work may in fact be wrong. The problem chiefly involves antibodies, a class of proteins scientists have used for generations for everything from quantifying receptor expression to purifying critical gene products.

Historically, scientists who needed an antibody against a protein would either make it themselves or get it from a colleague. Though this process is straightforward, it can be tedious and slow, and with the rise of genomics and proteomics, such an artisanal approach could no longer produce the large numbers of different antibodies researchers needed. In response, an antibody production industry arose to cater to the growing need. Unfortunately, the low entry barriers and potential for huge profits soon yielded a glut of dubious reagents. Now researchers are surveying the resulting damage and trying to decide how to proceed.

"I think it's a mounting problem, and it is ubiquitous, [causing] hundreds of millions of dollars to be wasted annually," says Mike Simson, president and CEO of **One World Lab** in San Diego, California, an antibody marketing company that aims to simplify the antibody validation process. "It's just gotten out of control at this point," he adds.

Light chain, heavy chain, supply chain

Unbeknownst to most scientists, many research-grade antibodies take convoluted routes to the lab. A few suppliers manufacture the antibodies they sell in their own facilities, but many of the largest vendors operate more like department stores. Just as a group of companies in Shenzhen, China, makes most of the world's electronic gadgets, a cluster of businesses in Wuhan, China, makes numerous antibodies. Reagent vendors buy these products, then put their own brand names and stock numbers on them to resell. As a result, a market that appears to offer a huge selection of competing products is in fact selling a much smaller set, rebranded in multiple catalogs.

Investigators often buy an antibody from one supplier, discover it doesn't work for their application, then order another antibody from a different supplier, not realizing that it's exactly the same product. With each purchase costing hundreds of dollars, researchers commonly waste thousands on these repetitive failures. Worse, when an antibody does work well enough for publication, the next batch of it may not, and scientists can't trace the problem. "When you don't have traceability, you don't have accountability, and then you don't have reproducibility—and without reproducibility you don't have science," says Simson.

To address that problem, One World Lab offers fully traceable antibodies from about 40 different manufacturers, and sells them in sample-size kits. Scientists can order a set of genuinely distinct antibodies against a particular target, test them all, and identify the one that's best for their purpose. Simson claims that this practice benefits antibody makers as well as scientists: "There needs to be this place where [the data] are coming back for that company to understand how their products are working." cont.>

"Clearly there is a need for cleaning up some of the reagents that are available for researchers."– Mathias Uhlén, director of the Human Protein Atlas project

Unfortunately, while researchers seem to value One World's service, they haven't been very good at paying for it. "What we've found in practice is that researchers are buying [an antibody sample kit], testing it, and identifying what works, then they're going directly to [the manufacturer] and buying it," says Simson. "They need to keep buying through One World Lab to keep this platform alive." Unfortunately, Simson's concerns were not unfounded; the company recently announced that it will be shutting its doors. In an announcement on their website, Simson stressed that although One World's business model needs adjusting, "transparency and affordability are vital to advancing science." It's unclear whether another company will have better luck capitalizing on that need.

Positive and negative selections

Besides calling for a more transparent supply chain, many experts in the field advocate establishing a more formal, industry-wide set of standards for validating these reagents. "Clearly there is a need for cleaning up some of the reagents that are available for researchers," says Mathias Uhlén, director of the Human Protein Atlas project, and professor and founder of the Science for Life Laboratory at the **KTH Royal Institute of Technology** in Stockholm, Sweden.

Uhlén and his colleagues had to confront the problem of antibody validation head-on while developing the Human Protein Atlas, which created thousands of antibodies to map the expression patterns of proteins throughout human tissues. Uhlén also helped form the International Working Group on Antibody Validation (IWGAV), which published its proposed guidelines last October (*1*). Around the same time, the **Global Biological Standards Institute** (GBSI) organized a meeting at the Asilomar Conference Grounds in Pacific Grove, California, and formed working groups to continue discussing the issue (*2*).

All of the new standards created by IWGAV and GBSI share two fundamental principles. "One thing [is] that any validation of an antibody has to be done in an application-specific manner," says Uhlén. An antibody that works well for Western blots on denaturing gels, for example, should not be presumed to work equally well in nondenaturing assays such as fluorescence-activated cell sorting. Second, the standards set up protocols or "pillars," defining exactly what tests an antibody must pass to be validated for a given application.

Each pillar employs a distinct technique, offering multiple ways to achieve validation. For example, researchers can take a genetic approach, knocking out or knocking down the gene for the antibody's putative target and confirming that this eliminates the antibody's binding sites. Another method uses the test antibody to purify the target protein, then confirms that protein's identity through mass spectrometry.

Though the different efforts to establish validation standards largely agree in outline, niggling disputes remain. One point of contention is whether validation should be considered "pass/fail," or whether it should yield a ranking to score different antibodies as better or worse than others for the same target and application. Uhlén favors the former approach, while GBSI has been leaning toward the latter.

As difficult as it is to get scientists to agree on the standards, applying them is even harder. Uhlén's group has been systematically testing all the antibodies used for the Human Protein Atlas to see if they meet the new standards. "The goal is that three to four, maybe five years from now, we will only have antibodies that have been formally validated with at least one of these pillars in the Protein Atlas; but this is actually a huge effort, and we are busily working on it," he says.

For the vast majority of commercially available antibodies that haven't yet been validated under the newly defined standards, end users may want to take matters into their own hands. In labs that don't already have gene-knockout or mass spectrometry capabilities, a novel RNA in situ hybridization method might help.

RNA hybridization has traditionally been a tricky technique, but **Advanced Cell Diagnostics** (ACD), a Newark, California–based subsidiary of Bio-Techne, hopes to change that. The company's RNAScope system uses proprietary probes and markers to quickly and consistently quantify RNA levels in a cell or sample. While RNA expression doesn't prove protein expression, investigators can use the correlation as an easy way to check their antibodies. "RNAScope is really allowing customers to validate RNA expression as a precursor to being translated to a protein, [to] confirm expression as an orthogonal method," says Chris Silva, vice president of marketing at ACD. RNA levels that diverge markedly from antibody-binding levels could provide an early warning of trouble with the antibody.

Investigators can also use RNA assays to narrow the scope of a project before ordering expensive antibodies. Silva says his company can deliver custom RNAScope probes for any sequence within a few weeks, which could help quickly identify targets worth studying with antibodies.

Keeping the hits coming

Though some commentators have described the current situation as a crisis, industry veterans see it more as a longstanding problem that's finally being noticed. "I don't think it's a new problem," says Alejandra Solache, vice president of new product development at **Abcam** in Cambridge, United Kingdom. "The new tools we have for

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antibody validation are actually making us more aware of some of the potential drawbacks or shortcomings of tools like antibodies that we have been using in the past," she explains.

Because researchers weren't paying much attention to antibody validation, manufacturers didn't always invest in it. "A lot of the cost of antibody development comes from the validation; that's possibly where some companies have been trying to save money and not put as much emphasis on that," says Solache.

For its part, Abcam aims to set a high standard for antibody production and testing. "It's our responsibility to provide products of the highest quality; and as new technologies become available for antibody validation, we are trying to use those technologies on a larger scale so we can better evaluate those products," Solache says. She adds that the company is already validating many of its antibodies with the same techniques Uhlén and his colleagues have advocated.

Abcam is one of the "hybrid" antibody suppliers in the market, selling a combination of antibodies they've developed themselves and those they rebrand from other companies. Solache insists that they select their suppliers carefully, and adds that calls for complete transparency in the supply chain are problematic—resellers commonly have contracts with their suppliers that forbid publicizing the relationship. "I think it isn't necessarily a problem if the quality of the antibodies is good," she says. "We need to sort out the quality first."

For researchers who just want to know where to buy an antibody, Solache recommends looking for companies that provide validation data. Reviews of particular antibodies, either on company websites or independent review pages, can also provide good insights.

While large suppliers like Abcam invest in new antibody validation technologies, smaller firms face their own problems, particularly those specializing in manufacturing. These companies must first decide what antigens to make antibodies against, then try to guess how they're likely to be used. "By the very definition of scientific research, it means someone is working at the cutting edge," says Jason Li, CEO of **Proteintech** in Rosemont, Illinois, a company that specializes in antibodies, ELISAs, and proteins. Li adds that "scientists need an antibody reagent for their own cutting-edge research, [and] nobody has used that antibody for that purpose before. That's actually a very difficult thing for a manufacturer to predict, because nobody can predict science."

This conundrum has made antibody manufacturing similar in some ways to the movie and music industries. Antibody makers speculate on what antigens are likely to be popular targets, invest in making and validating antibodies against them, then see what sells. Li estimates that about 1 in 10 antibodies will be a "hit," with the other 9 languishing in a company's freezers. The hope is that the profits from that single hit can fund the development of the next 10 antibodies and keep the cycle going.

Multiple factors have caused that model to falter in recent years. Li cites the growing cut distributors have taken from manufacturers' profits, and the departure of investors who'd previously sunk money into antibody makers in the hope of quick returns. As budgets shrank, it was easy to see where to make cuts. "It costs two-thirds of the money to validate an antibody, and it only costs one-third to make one," says Li. The opaque supply chain exacerbated the problem, preventing researchers from identifying where bad antibodies were coming from.

"The only way to [fix] that is transparency," says Li, who envisions a future where "each antibody is thoroughly communicated to the researchers—where it was made, how it was made, what it can be used for, and what its limitations are."

Change starts at home

While antibody makers and sellers struggle with transparency and standards, researchers also need to change their approach. "There are good antibodies and bad antibodies, and there are people who know how to use them and people who don't necessarily know how to use them properly," says Roberto Polakiewicz, chief scientific officer of **Cell Signaling Technology,** a research and diagnostics products company based in Danvers, Massachusetts.

Polakiewicz explains that many experienced scientists already know how to approach commercial antibody validation—by looking for data. Trustworthy antibody suppliers now routinely provide data showing how each antibody in their catalog has been validated. Labs with the capability to do their own tests can also check the antibodies they've ordered. The specific protocol one plans to use in an experiment should also inform the purchase. "When you're doing immunofluorescence, for instance, if you're using the wrong fixation process or fixative you could really get the wrong results even with a great antibody," he says.

Good antibody hygiene continues even after an experiment yields publishable results. "If you intend to reproduce a publication, it's important that all the methods are there [and] are very detailed," says Polakiewicz, but he adds that researchers' track records in this regard have been "very uneven." Papers that fail to report sources and lot numbers of the antibodies used are inherently hard to reproduce.

Lot numbers are particularly important and commonly ignored; as a biological product, antibodies can easily vary from one batch to the next. Good suppliers are happy to describe their quality control procedures, but for maximum reliability researchers should try to do an entire series of experiments with antibodies from a single lot.

For both antibody suppliers and researchers, the ultimate solution to the antibody validation problem will be to rely on the fundamental principles of research. "In the end it's really more about doing good science. Developing an antibody and validating it is not different from doing an experiment correctly, and doing the right experimental design with the proper controls," observes Polakiewicz.

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