Antibodies have been identified as contributors to a crisis in reproducibility. Antibody quality has changed over time, but so has the approach of many researchers. Antibodies are not inherently good or bad. It is how they are used that creates a potential crisis.

We need to understand the context in which “fit for purpose” antibodies bind targets in specific assays. We need to consider whether antibody performance has deteriorated over time. And we need to become familiar with new detection methods and strategies that can uncover existing antibody pitfalls.

Immunassays such as immunohistochemistry and Western blotting were report-ed over a half century ago. Today’s advanced techniques—ELISA, qPCR, ChIP sequencing, nanoscopy, and nanoimmunooassays—push the limits of antibody performance.

Antibodies as Commodities

Before 1960, scientists handcrafted their own polyclonal antibodies for research. Antibody development was an art. By the early 1960s, Cappel Laboratories and other pioneering companies started making secondary antibodies for research while promoting their antibodies that produced antiserum and antibody technology, the art of antibody development, validation, and marketing have changed. Arguably, so has the validity, quality, and reproducibility of the data collected using the new antibody tools.

But researchers are also partly responsible for antibody-related data quality problems, perhaps because of time pressures—publish or perish—that exist in academia. Rather than take time to investigate antibody performance, some researchers take shortcuts and purchase antibodies based on the first result appearing in an internet search, without considering if an antibody is “fit for purpose,” meaning whether or not an antibody will perform according to the needs of a specific experimental condition.

Others make selections based on the reputation of a company, or the high cost of an antibody, assuming that if the cost is high so is the quality. The antibody’s ability to recognize the correct target species, domain, or post-translational modifications may be overlooked. Another problem is the common assumption that an antipeptide antibody screened by Western blot will recognize a native target. For many researchers who have developed a strong sense of outsourcing antibody technology, the art of the science has been lost.

A symbiosis exists between researchers needing antibodies and companies that market them. A researcher could contact the supplier to determine if an antibody is suitable for its intended use. But if the antibody supplier is one of the many companies that only sells antibodies—and never develops or validates them—then it will probably lack the ability to answer technical questions.

Ideally a company is involved in all aspects of the process, including antigen design and production, antiserum production, antibody purification, and testing using multiple immunoassays. Such a company is best suited to guide users and provide researchers with efficient and reliable experimental tools.

An Inconvenient Solution

It has been argued that traditional polyclonal and monoclonal antibody development should be replaced by more recently developed recombinant technologies, which rely on combinatorial powers of expression through cloning for antibody development and screening. But these technologies are better suited for biopharma and the clinic rather than for research. The critical flaw

Antibodies, once “hand crafted” for research applications, are now commodity items. As antibodies came to be produced in ever-greater quantity, and as they came to be used by ever-busier professionals, reproducibility became an issue. To improve quality, demanding recombinant approaches have been proposed, but simply paying more attention to monoclonal and polyclonal basics may suffice.
in these technologies, as far as research is concerned, is their relative complexity. The production of polyclonal antibodies is easier, less expensive, and faster.

For example, investigators still demonstrate the biological significance of putative post-translational modifications by producing multiple antibodies taking a shotgun approach. Function is then confirmed by validation using the appropriate assays. This is easily accomplished when the resource burden of producing these antibodies is low.

Recently, advocacy of recombinant technology was questioned in an article posted on the CiteAb website. The article’s comment thread, in particular, highlighted the need to perform and understand reagent validation procedures. We add that reagents must be validated as fit-for-purpose reagents, in that validation by one or more assays does not imply functionality in all assays.

**Resolving the Antibody Dilemma**

The antibody dilemma presents two less-desirable alternatives: 1) continue to use antibodies of uncertain quality, or 2) use demanding molecular alternatives to clonal antibodies. This dilemma, however, can be resolved. The solution is for both researchers using antibodies and companies selling antibodies to understand the process of validation and embrace the concept of fit-for-purpose reagents. We need to stop taking so many shortcuts and relearn some of the art behind antibody science.

One shortcut some researchers take is to omit controls. Western blotting is often used to assess antibody quality by identifying specific post-translational modifications such as serine phosphorylation. Without the appropriate positive and negative controls, Western blotting has dubious validity. When performed properly, however, it becomes a convincing method to demonstrate antibody specificity and overall quality.

Negative controls can include peptide inhibition assays in which the antibody is preincubated with immunogen; in vitro phosphatase treatment of phosphorylated protein; point mutation procedures that change serine to alanine; and CRISPR, siRNA, or knockout cell lysates. Positive controls can include comparison of stimulated versus unstimulated cells after activation by an inducing agent; the inclusion of lysates from cells known to express the target; and in vitro phosphorylation by the target’s kinase. One consequence of not using proper controls is that antibodies may detect signal where antigen is not present.

Another shortcut is insufficient scrutiny of research reagents prior to purchase, especially if you are depending on technical support. Many companies that sell antibodies outsource all aspects of antibody development and testing through a process called OEM (original equipment manufacturer) supply. Simply put, OEMs sell products from other companies under their brand without divulging the source of the antibody.

There are good OEMs, and there are bad OEMs. A good OEM has a stringent quality assurance program and insists on validation data and lot-to-lot consistency and supports the sale of these products through knowledgeable technical representatives. A bad OEM is promiscuous, sells as many reagents as it can, and brokers deals without regard to performance or technical support.

Antibody-producing companies may also take shortcuts. For example, some producers neglect quality assurance. This happens because making antibodies is easy, whereas demonstrating that they work is hard.

Many scientists have explained that they might purchase a dubious antibody if it happens to offer low cost and immediate availability. Such an antibody might just work.

We believe this approach and other approaches that rely heavily on outsourcing antibodies as commodities have hurt not only the antibody industry, but also the research community. We should shun these approaches and close the credibility gap that they have opened.

**Antibody Haste, Research Waste**

The rush to market for some companies acknowledges the high operational costs and difficulties in producing antibodies. Now that antibodies against relatively easy targets are already available, reagent development is taking on a greater challenge—increasingly stringent sensitivity and specificity requirements.

Making a good antibody does not ensure it will actually sell, or cover the expense of reagents that do not pass rigorous testing criteria. Companies do not possess crystal balls predicting which new reagents should be developed. Few antibodies developed are profitable. Rather than rush to market, an alternative approach is to slow down and embrace the relationship between users and producers. Collaborations between researchers and antibody-producing companies are fruitful if both parties are mindful of each other’s objectives and timelines.

Reproducibility of antibody performance can be maximized by antibody manufacturers when well-defined acceptance criteria are established and performance results of newly produced batches are compared to multiple historical batches. Researchers need to invest more time in discovering the performance characteristics of antibody reagents. Validation of fit-for-purpose reagents will result in high-value data collection.

Don’t blame the antibodies for poor reproducibility. Blame the shortcuts often taken by researchers and antibody manufacturers. Polyclonal and monoclonal antibodies are alive and well.

**References**