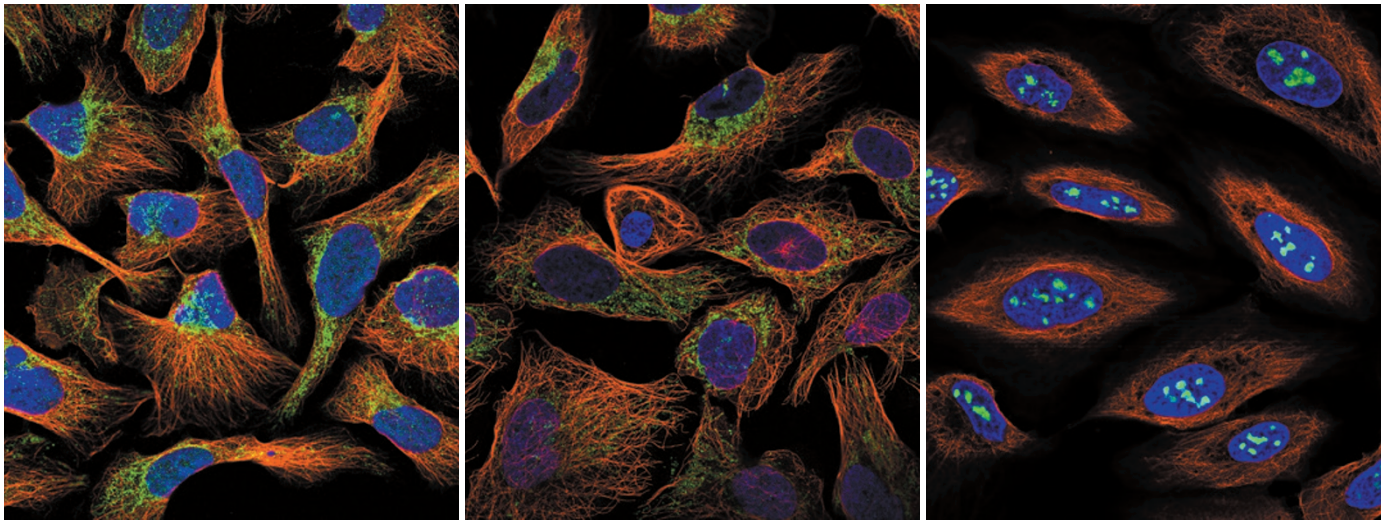
HUMAN PROTEIN ATLAS, WWW.PROTEINATLAS.ORG



Three antibodies (green) against the same mitochondrial protein. The unexpected pattern on the right shows the third antibody binds an unintended protein.

Information is beginning to accumulate. More than two dozen web portals have sprung up to help researchers select antibodies. Some collect user reviews on antibody performance and offer comparison tools. The Antibody Validation Channel, a project of the scientific publisher F1000, allows researchers to post their accounts and even request peer review. Biocompare has hired a content editor whose sole focus is to reach out to the research community and get them to write reviews.

Some antibody suppliers, such as St John's Laboratory in London, offer researchers free products in exchange for testing and sharing the results. Antibodies-online, a market place for antibodies, arranges for an independent third party to perform validation. At Antibodypedia's knockdown initiative, launched in September, life scientists can earn hundreds of dollars in free reagents if they submit data showing that gene-silencing reagents such as small interfering RNA or CRISPR–Cas9 eliminate an antibody signal for a given target.

But many scientists are wary of information from anonymous reviews. Data supplied by both users and companies can be sparse, and some projects share data only if they confirm that an antibody works as expected. "Sometimes it seems easier to hire a detective than to order a specific antibody," concludes an overview of antibody portals[5].

FUTURE ASSESSMENTS

Some researchers are developing mechanisms to compare antibodies directly. Aled Edwards at the University of Toronto, Canada, is director of the international Structural Genomics Consortium (SGC). He and his SGC colleagues used mass spectrometry to detect and compare the sets of proteins pulled down by immunoprecipitation with more than 1,000 antibodies[6]. The collaboration ran across 5 reference laboratories, took 4 years and cost US$3 million, not counting in-kind donations. Ultimately, it established a procedure to score antibody quality and share quantitative information about its performance, specifically for 'pull-down experiments', in which proteins are pulled out of solution using antibodies.

Fridtjof Lund-Johansen, a proteomics researcher at Oslo University Hospital in Norway, is developing an ambitious bead assay that tests thousands of antibodies at once[7]. The plan is to separate cellular proteins into many different fractions, then profile the proteins in each fraction using two different methods. One is mass spectrometry and the other is a bead-based array with thousands of antibodies. The mass spectrometry data serve as a reference for the results obtained with antibodies. Turning the idea into a refined assay will take considerable work, Lund-Johansen admits. "It is extremely ambitious. It is totally crazy, but it is the only way to go." Other scientists are intrigued at the approach but wonder if it will predict antibody performance in common techniques.

Blanket assessments of antibodies can be overinterpreted, says Ulf Landegren, a proteomics technology developer at Uppsala University in Sweden. "It is far more meaningful to discuss the ability of assays to detect the correct protein, rather than whether antibodies or other binders bind the right protein." A case in point is cross-reactivity, when an antibody binds proteins other than its specified target. Cross-reactivity depends not just on a particular antibody, but also on the complexity of a sample, the concentration of the antibody and the rarity of the target protein. He recommends that rather than relying on a single antibody, researchers should instead test antibodies in pairs that are designed to bind to different parts of a target protein. Parts of a sample labelled with both reagents are less likely to represent off-target binding.

One problem with this approach is that it is hard for scientists to know if they are purchasing different antibodies. Vendors often obtain products from different sources and are not required to disclose the original manufacturer. As a result, researchers who want to compare several antibodies may end up comparing identical products sold by several vendors. A handful of companies, including Genlogica and One World Laboratories, both in San Diego, California, only sell products labelled by the original manufacturer and offer 'trial size' antibody batches so that researchers can test products side by side in their labs.

The toughest challenge is not so much in antibody characterization but in persuading cell biologists to hold back on using antibodies until these are thoroughly evaluated, says Edwards, although he doubts that scientists will become savvier unless funders and publishers force the issue. "Right now we have an unregulated market, where you don't have to have any quality to sell your product." In other words, he says, guidelines, characterization data and conscientious vendors only matter if researchers invest effort into selecting reagents. ■

Monya Baker *writes and edits for* Nature *in San Francisco, California.*

1. Saper, C. B. & Sawchenko, P. E. *J. Comp. Neurol.* **465,** 161–163 (2003).
2. Bradbury, A. *et al. Nature* **518,** 27–29 (2015).
3. Bordeaux, J. *et al. BioTechniques* **48,** 197–209 (2010).
4. Vasilevsky, N. A. *et al. PeerJ* **1,** e148 (2013).
5. Pauly, D. & Hanack, K. *F1000Research* http://dx.doi.org/10.12688/f1000research.6894.1 (2015).
6. Marcon, E. *et al. Nature Methods* **12,** 725–731 (2015).
7. Wu, W. *et al. Mol. Cell. Proteomics* **8,** 245–257 (2009).
8. *Nature Methods* **12,** 373 (2015).
9. Conze, T. *et al. Glycobiology* **20,** 199–206 (2010).

CORRECTION

The Technology Feature 'Connectomes make the map' (*Nature* **526,** 147–149; 2015) misnamed the MultiSEM model and gave the wrong citation in reference 3. MultiSEM 505 should have been Zeiss MultiSEM, and ref. 3 should have referred to Zingg, B. *et al. Cell* **156,** 1096–1111 (2014).