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PERSPECTIVE

Commercial Antibodies: The Good, Bad, and Really Ugly

John R. Couchman

Biomedicine Institute, University of Copenhagen, Biocenter, Copenhagen, Denmark

SUMMARY The range of antibodies available commercially grows ever larger. Perhaps as a consequence, quality control is not always what it could and should be. Investigators must be aware of potential pitfalls and take steps to assure themselves that the specificity of each antibody is as advertised. Additionally, companies should provide the necessary information about the antigen and antibody to investigators, including references, so that the appropriate controls can be included. **(J Histochem Cytochem 57:7–8, 2009)**

"LOOKS LIKE WE bought another vial of PBS" said my postdoc the other day. He was referring to a Western blot on his bench that was remarkably clean—no background, but no polypeptides either, not even his positive control. This was a frustrating end to a frustrating week for my postdoc, not least because an earlier vial of the same antibody, from the same source, had worked fine. However, the company concerned has established quite a reputation for dubious quality control. I remember not long ago recommending an antibody to a colleague by e-mail, available from this same company, and he quickly shot back to me with a comment that he avoids this company like the plague. To paraphrase John McEnroe, "Was I really serious?" I wondered whether he thought I was trying to slow up his progress by suggesting this route. On another occasion, I was talking to some colleagues in the United Kingdom not long after giving a seminar at their institution and mentioned an antibody I considered not worth wasting money on. "Let me see if I can guess which company" came the reply. Spot on—he got it right the first time.

So I asked my colleague in the laboratory whether he was going to phone the company and complain. To my surprise, there was some reticence. Maybe if it was his grant funds and not mine, he'd be more energized! However, I think I know the real reason. Many of these companies have their telephones manned by experienced operators who know how to fend off callers by questioning whether the complainant really knew what he/she was doing. My postdoc is very experienced, yet perhaps given to more than a touch of self-doubt. "Did I really use the right dilution of secondary antibody? Were the peroxidase substrate reagents okay?"

Correspondence to: John R. Couchman, Biomedicine Institute, University of Copenhagen, Biocenter, Ole Maaloes Vej 5, 2200 Copenhagen N, Denmark. E-mail: john.couchman@bric.dk

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What the Researchers Can Do

We are in an era of "off-the-shelf" molecular and cellular biology. There's a kit for everything; how many laboratories would know how to do a cDNA mini-prep from scratch? Similarly, there are commercial antibodies out there against just about everything. There was a time when, if you got interested in a molecule, you made an antibody yourself. You had to characterize it also. Many of my past students have learned to do this, but no more. In this fast-paced, competitive environment, there is no time to make the antigen, wait for the rabbit to do its stuff, and characterize the product. More usually, affinity purification was a required step. I can remember well a reviewer of a manuscript many years ago insisting that not only must I affinity purify the antibody but also perform antigen adsorption on my tissue sections. Quite right too. These days, however, we take all this on trust. If the label on the vial says rabbit anti-protein kinase Cβ, then that's what it is. Some students easily have faith in the written word; how many of us have trouble explaining in journal club that just because Smith and Jones show data that support the idea that the phosphatase PTP33 is upstream of protein kinase Z, that it actually is so. Healthy skepticism is healthy, and every reader has surely seen published data with antibodies that just do not look right.

We cannot characterize every antibody we buy, surely? Granted, many commercial antibodies are just what they say they are. The saving in time, energy, and perhaps money is enormous (although many are exor-

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bitantly expensive!). Cadging antibodies from your friends is much cheaper! How to spot the bad ones? This might not be as easy as it sounds. Some monoclonal antibodies will not recognize the denatured protein on a Western blot, so this simple expedient of checking that a protein of the "right" mass is detectable (preferably a purified or recombinant protein) is not always available. For many antibodies, however, this is at least a suitable precaution. If immunohistochemical staining of, say, liver is the aim, at least a Western blot of a liver homogenate to check for appropriate polypeptide recognition, and just as important, the lack of "nonspecific bands" can and should be done. If your protein is 50 kDa, a slew of polypeptides recognized between 100 and 200 kDa should set alarm bells ringing. However, deparaffinized archival sections, subjected to hot citrate antigen retrieval, can throw up new, potentially misleading cross-reactivities that would not be seen by Western blotting a fresh lysate of liver straight from the animal. Fresh human tissue is, in any case, just not a practical approach for most of us. However, I have noticed that antipeptide antibodies are particularly prone to being badly behaved on tissue sections.

In many instances, common sense and experience come to the rescue. If we are studying an extracellular matrix molecule and our antiserum picks out nuclei like beacons, it is time for some healthy skepticism. Many investigators use multiple antibodies from different sources and compare staining patterns. This is a good approach, particularly if they are a mix of monoclonal and polyclonal antibodies and especially if they recognize different epitopes on the antigen in question. Of course if the antigen has a well-described distribution in a particular tissue or cell type, this is easily checked. Antigen adsorption, where the antibody is premixed with the appropriate purified antigen before application to the tissue sections or cells, seems to be a dead art. It is a powerful way to look for nonspecific reactivities. In many cases, this is understandable; sufficient purified antigen may not be at hand. Unfortunately, many commercial antibodies are not affinity purified from serum. It is quite common that a polyclonal antiserum will not only recognize the antigen in question but other proteins in addition. This is usually quite clear in Western blots and leaves no clear way forward except affinity purification. With a commercial antibody, this can be expensive, and then it is time to get on the telephone and start a constructive conversation.

At least with monoclonal antibodies, as long as the cloning process itself was performed efficiently, only one antigen epitope is, in general, recognized. If the antibody recognizes the denatured antigen on Western blots, life becomes easier for quality control. If not, the options for verifying specificity are more complex, for example, immunoprecipitation or, again, antigen adsorption. If the epitope is a protein, and an appropriate cDNA is at hand, in vitro transcription/translation followed by immunoprecipitation is one way forward. This is not a Friday afternoon experiment, however; it takes some time. A very powerful approach can be applied where knockout cells or animals are available, which should provide a clear negative control for staining, Western blotting, or another technique. Similarly, if the antigen is known to be absent from a cell or tissue type (e.g., green fluorescent protein that is not normally present in vertebrate cells), this is another easily performed check.

What the Companies Should Do

For monoclonal antibodies, companies can help out a great deal by providing references that show a clear antibody characterization. Most companies do not make their own antibodies but license them from researchers. With monoclonal antibodies defined in a clear way, this should provide a high degree of confidence to the user. Polyclonal antibodies, by their very nature, cannot be handled in the same way. Every new batch should be quality controlled, but in the real world this does not happen. I would suggest, however, that these companies be open minded to problems.

In 2005, Dr. Clifford Saper (Editor-in-Chief, *Journal of Comparative Neurology*) wrote an editorial in the form of an open letter to contributors about antibody problems (Saper 2005). It is worth a read and is full of sound advice. Particularly striking is an admonishment not to touch any antibody where the antigen is not clearly stated. Sometimes for "proprietary" reasons, details on the antigen are withheld. This is not good science, and we should boycott those antibodies. How could such an antibody be satisfactorily characterized? Equally, it is up to us in our manuscripts to clearly state the source, product, lot numbers, and the methods of characterization for each antibody.

Our own experience is that one batch of an antibody may work fine; the next, with a different lot number, may not. This is frustrating and an expensive waste of time. Some companies are quick to work with investigators to remedy the problem, but some are not; the sale is everything. Certainly, where a positive control lysate or similar is provided with the antibody, this can be a valuable way to gain confidence. Why did my postdoc have batch problems? We do not know; maybe the failed batch spent too long out of the freezer before dispatch, but some companies should recognize they have a bad name for quality or after-sales troubleshooting. Therefore, in the imperfect research world, some companies are good and some could try harder. In the end, it is up to the researcher to have his or her eyes wide open.

Literature Cited

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