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The antibody horror show: an introductory guide for the perplexed

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ABSTRACT

The biological literature reverberates with the inadequacies of commercial research-tool antibodies. The scientific community spends some $2 billion per year on such reagents. Excellent accessible scientific platforms exist for reliably making, validating and using antibodies, yet the laboratory end-user reality is somehow depressing – because they often "don't work". This experience is due to a bizarre and variegated spectrum of causes including: inadequately identified antibodies; inappropriate user and supplier validation; poor user training; and overloaded publishers. Colourful as this may appear, the outcomes for the community are uniformly grim, including badly damaged scientific careers, wasted public funding, and contaminated literature. As antibodies are amongst the most important of everyday reagents in cell biology and biochemistry, I have tried here to gently suggest a few possible solutions, including: a move towards using recombinant antibodies; obligatory unique identification of antibodies, their immunogens, and their producers; centralized international banking of standard antibodies and their ligands; routine, accessible open-source documentation of user experience with antibodies; and antibody-user certification.

Today's biological sciences are dependent on reliable antibodies. A typical biology laboratory spends from €5000–10000 a year on commercial research tool antibodies – but many spend 10′s to 100′s-fold more, and use more than a 100 antibodies routinely, and use them on a daily basis [1]. In this article I refer specifically to such commercial research tool antibodies, not to therapeutic or diagnostic antibodies: these are rigorously defined. An estimated $2.5 billion per year was spent on research tool antibodies in 2015 [1]. In a perfect world, we would like to have, off-the-shelf, affinity binders that specifically bind to all possible epitopes, as exposed in all conceivable cell biological and biochemical technologies. But the world is imperfect, so we have instead commercial research-tool antibodies (c-Abs). I recently re-discovered a bit of journalism I wrote long ago complaining about the depressing quality of reagents [2]. From my old lab notebooks, I can now reveal the reagents in question: commercial antibodies. Was this the first "official complaint" on this topic in the literature? Well, it was in a minor non-peer reviewed journal, so I am not surprised if you did not spot it. Still, I claim it as justification for this note. Since 1989, my moan has loudened to a faint but sustained scream: in short, my current status is abject terror. I am scared of c-Abs, of their existential impact on our biological endeavour, and of their effects on the "reproducibility crisis" [3]. And, to borrow from a recent tune, "I know I'm not alone". For there is a long, long and increasingly long line of excellent and profoundly depressing comments, recommendations and studies on the various deficiencies in the validation, characterization, and in the production, supply and use of c-Abs, and how this might be – no let's cut to the chase – *is* polluting the scientific literature [4–8]. Not to mention a Dark-Web of unreported, unpublished studies classified as "did not work", or "company confidential", lying silent in computer files and desk drawers around the world, the harvest of dysfunctional c-Abs. Much of this waste is being financed from the public purse. It all sounds like a recipe for disaster.

It seems ironic that this situation has developed. For example, given the outstanding scientific quality of the Alpbach symposium 2017 (http://affinityproteomicsalpbach.com/index.php). We well know how to make and to characterize affinity protein binders. So what exactly is the problem with those c-Abs that we seek when we need, for example, to track unfamiliar biochemical pathways, localize unfamiliar target proteins, or quantitate changes in a phosphorylated site in response to an experimental manipulation? And, the target being unfamiliar and there being no colleague who can offer a reagent, we turn to The Catalogues – and – Oh joy! there are so many antibodies to help us here.

In fact, Andrew Chalmers, of the antibody cataloguing site CiteAb [9,10], has collected over 3.8 million (3.8×10^6) discrete c-Abs in catalogues and cited in the scientific literature. Only 15 years ago Michaud et al. were elated to find "approximately ten thousand antibodies" available from commercial sources [11]. And even 5 years ago, The Scientist magazine reported an increase of only 5000 c-Abs per year [12]. Yet, in the 15 years since the Michaud et al. article, the increase in c-Abs has slightly beaten Moore's law (i.e. a doubling every 18 months). Where have they all come from? Perhaps coincidentally, the start of the explosive growth coincided with the "completion" of the human

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genome project in April 2003. Suddenly there were an awful lot of open-reading frames with no known function, in need, as it were, of protein binders – and the c-Abs scuttled in. Pushing four million antibodies is really being spoiled for choice, one would think; that is one antibody-per-protein for some 150 human-proteome-size species. Sounds like a lot. Of course, we often need several antibodies for each protein: antibodies to quantify a level of phosphorylation may not bind in solution, or on paraffin sections, or on a Western blot, etc. Then there are issues of detecting differential splicing, glycosylation, conformers, and so on. Then the antibodies might be directly labelled with many different tags and for cytometry, with diverse fluorochromes, etc. At a generous 10-antibodies per-protein-per-proteome, 4 million antibodies could hypothetically provide proteome-wide binders to cover about 15 human-like species. But after us, mice, rats, Drosophila, dogs, Zebrafish and *C.elegans*, the give-me-a-proteome-of-binders wish-list begins to thin out substantially. Seven full genomes covered, plus small-change. c-Abs sufficient, in theory, for 5×10^5 antibodies per popular genome. However, popular targets acquire a notable aura of more-than-several c-Abs, while other targets are neglected. For example, currently 6542 antibodies in CiteAb target "EGFR", 5140 targeting the human receptor, and 10993 target "histone H3" (see Table 1). Even allowing for primary antibodies labelled, for instance, with many different fluorochromes, targeting variously phosphorylated forms etc. this seems to really satisfy any possible market.

To cut this short: there seem to be a lot of c-Abs out there, so what is the problem? We read that it is a not unusual situation for a PhD student or post-doc to find that an antibody "does not work" [6,7,13]. In fact, even in those hands, calloused and scarred by decades of cell biological and biochemical toil, many c-Abs "do not work" [4,13]. So why is that, I wonder?

For myself, although I have continued to have had routine problems with c-Abs, I really did not give it too much thought. It was a bit like continually being attacked by gnats, irritating but not too serious. Just one more slice off the budget – just another c-Ab from The Catalogues. I was not really interested in antibodies *per se*, sorry about that, I just wanted working c-Abs. But often they did not work. Let me be more specific about that: using the techniques that I applied, the antibodies often failed to specifically bind something resembling the target they were claimed to react with − they were not fit-for-purpose (F4P). I guess possibly some one in three functioned. Reviewing my colleagues, I find this is a common experience. The antibodies I made and characterized myself, and those my colleagues extensively validated: those did work as predicted.

When I got in touch with the commercial entity concerned and told them the reagent they had sold me did not work, I generally heard "Are you using it correctly?". This was a perfectly valid response, as a survey initiated by participants at Alpbach and the recent Asilomar-Global Biological Standards Institute (GBSI) meeting (https://www.gbsi.org/event/antibody-validation-2016/) showed recently: only 43% of early career researchers (< 5y tenure) validated c-Abs, and 31% did not validate, or worse, *saw no need* to validate c-Abs at all [1]. Mercifully, the perceived and reported need to validate antibodies before using them increased with time in tenure − or perhaps it was only the people who validated their antibodies that got any further in their careers? I do not think that point has been investigated yet. The major reason for *not* validating antibodies was given as "it takes too much time". So companies are entirely right to ask whether you are using the antibody correctly. However, that is clearly not the only fly in the ointment, or irrelevant band on the Western blot, as it were, because it does take a lot of effort, time and skill to validate an antibody properly [14,15].

However, I had many antibodies that *did* work, so obviously I was, in general, using them correctly. What is more, I had come out of a laboratory where I, and everyone else, was routinely hung out to dry if they did not demonstrate clearly that their antibodies were or were not working correctly. So, yes, I was definitely using the antibodies correctly. And, no, they were definitely not working as advertised. What was going on with the c-Abs I just cannot say. Time went by, in fact, over 30 years went by in this state. After all: it was probably just me, wasn't it? One insect bite after the other, through the years. In fact, things got worse, because I started doing a lot of paraffin immunohistochemistry (IHC), and there the antibody situation was more of a dog-bite than a gnat bite. For example, we screened 28 monoclonal c-Abs to FoxO3a for specific reactivity on paraffin sections, without success, though things have improved recently here. Or take one of my favourites, alphavbeta6 integrin, a well-known membrane protein. To be polite, few of the c-Abs suggested to react in IHC with this target showed a staining pattern related to the distribution of the molecule. But then I happened across the Bradbury and Plückthun (and the many co-signatories) call-to-arms in Nature two years ago [16]. After carefully reading the citations, red lights started flashing. Clearly we need to do something.

There appear to be several reasons for the disconnect between what happens when we generate our own affinity binders, and what seems to be the often-unhappy everyday user-experience with c-Abs. These can be listed as (1) the reagents, (2) the targets, (3) the producers, (4) the users, (5) the literature and (6) training. Not to mention filthy lucre: the c-Ab market is around $2 bn in the USA alone [12]. I am not going to reiterate the many excellent in-depth discussions of these topics: I am just going to comment.

1) The reagents. We can expect c-Abs to be clean, selective, specific, intact, and reproducible, can we not [5,8]? Or at least identifiable [8]? Production, supply and, notably, identification sit firmly in the lap of the producer. Or, it seems, in the lap of the gods [6,7]. For sometimes, a critical reagent is not there, having been "disappeared" from the catalogue ("the rabbit died"; "we lost the clone"). For example, three most cited anti-EGFR (and EGFR-P) antibodies out of > 6300 identified on CiteAb are rabbit and goat polyclonals (Santa Cruz biotechnology: sc-03, −03-G and −12351), together cited over 1000 times, but now no longer accessible having been removed from the catalogue [17]. Does that not immediately make those 1000 publications irreproducible? And what of the remaining 2.2 million (2.2×10^6) rabbit polyclonal antibodies indexed by CiteAb (vs. 0.9 million monoclonals)?

I find even this single example is enough: Bradbury, Plückthun et al. are clearly right − the sooner we migrate towards completely transparent recombinant reagents, the better [16,18,19]. True enough, recombinant antibodies are currently costly to make. And the road will indeed be long – only ~5500 recombinant antibodies are as yet indexed by CiteAb. But clearly it was both costly and long to develop

Table 1
Some hyper-popular commercial tool-antibody targets.

Antibody Search Term[a]	Antibodies found[b]	Mab	Pab
Actin	18298	4789	13275
p53	16662	4558	11705
Histone H3	10993	2423	8406
Tubulin	9335	3491	5701
RAC	8281	1960	6155
EGFR	6542	2330	3947
Estrogen Receptor	6398	1484	4788
Fibronectin	5715	2665	2948
TGF-beta 1	5461	1152	4249
cdk1	3989	991	2906
VEGF	3341	1119	2175

Individual antibodies recovered from the CiteAb [10] internet site, using the Antibody Search Term shown, at mid-October 2017. Total antibodies indexed and their distribution by monoclonal (Mab) and polyclonal (Pab) reagents is shown.
Note: a) the Antibody Search Term recovered several different targets. So "actin" recovers alpha-, beta- and gamma-actin, for example. b) individual antibodies may be present in many forms, e.g. labelled with multiple fluorochromes: each form is counted.