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OPINION ARTICLE

REVISED The Antibody Two-Step Solution [version 2; peer review: 3 approved]

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Abstract

Problems with antibody quality have been described in numerous recent publications. In the present commentary it is argued that these quality problems are due primarily to issues of antibody variability and antibody validation. Further it is argued that the problem of antibody variability must be solved before validation can be useful. A two-step solution to the antibody problem is thus proposed.

Keywords

Polyclonal, Monoclonal, Antibody, Variability, Validation, Pooled Serum

This article is included in the **Antibody
Validations** gateway.

Open Peer Review**Approval Status** ✓ ✓ ✓

	1	2	3
version 2 (revision) 22 Dec 2015			
version 1 15 Sep 2015	✓ view	✓ view	✓ view
1. C. Fernando Valenzuela , University of New Mexico, Albuquerque, USA			
2. Andrew D. Chalmers , University of Bath, Bath, UK			
3. Angus C. Nairn , Yale School of Medicine, New Haven, USA			
Any reports and responses or comments on the article can be found at the end of the article.			

Corresponding author: Mike Browning (mdb@phosphosolutions.com)**Competing interests:** MDB is majority owner and CEO of PhosphoSolutions LLC, an antibody manufacturer.**Grant information:** The author(s) declared that no grants were involved in supporting this work.**Copyright:** © 2015 Browning M. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.**How to cite this article:** Browning M. **The Antibody Two-Step Solution [version 2; peer review: 3 approved]** F1000Research 2015, 4:810 <https://doi.org/10.12688/f1000research.7055.2>**First published:** 15 Sep 2015, 4:810 <https://doi.org/10.12688/f1000research.7055.1>

REVISED Amendments from Version 1

I have added a reference to the RRID process as an additional way to identify antibodies. I added a sentence explaining this reference.

See referee reports

In the past year there have been a number of articles in *Nature* and other major journals that discuss the antibody problem in somewhat apocalyptic terms¹. In my mind there are only two main issues in the antibody problem: antibody validation, and antibody variability. Both of these issues have straightforward solutions that do not require any massive influx of cash or massive restructuring of antibody production.

Antibody validation is the hardest nut to crack and causes the most confusion. There is no consensus on what constitutes suitable validation and this is complicated by the different methods for antibody use. However, antibody validation is a process like all science where knowledge increases as more and more work is done with the antibody. Many journals now insist that antibody validation data be provided and this is a key part of the solution. As long as the data are clear and the methods used adequately described, progress in validation will occur with time. However, none of this progress will matter unless we deal with the antibody variability problem. What difference does it make if an antibody is validated if it is not possible to obtain the same antibody for future work?

There are two main reasons for the variability in an antibody's performance. The first is that once an antibody is found to have a high demand, many different antibody manufacturers will try to make their own version of the antibody so they can sell it. But all these new antibodies will differ in unknown and unpredictable ways from the original antibody. Thus validation done on the original antibody may or may not be true for the new antibodies. One way to deal with this problem was recently suggested by Andrew Chalmers and his colleagues². They argue that all publications using commercial antibodies should report the name of the supplier and the catalog number of the antibody used. That way even if a supplier sells many varieties of the antibody a researcher will be able to order the same antibody that was used in the publication. Subsequently Bandrowski *et al.*³ proposed an even more detailed and efficient

antibody identification protocol with their Research Resource Identifiers (RRIDs) which are based on accession numbers assigned by an authoritative database. These suggestions are being incorporated into the instructions to authors in more and more journals.

Even though this action would greatly improve the value of antibody validation, an additional source of antibody variability would remain, namely lot-to-lot variability. This variability occurs because even if one buys the same antibody with the same catalog or RRID number, one still often encounters large variability in different lots of the same antibody obtained from different bleeds of the same animal or bleeds from different animals. There is a very straightforward fix to this type of variability. The solution is to pool all the positively screened serum collected from the animals. Virtually all lot-to-lot variability can be eliminated for polyclonal antibodies if this procedure is used. The antibody manufacturer could simply label the antibody as "pooled serum" to denote this fact. If this procedure is followed, it will no longer be necessary to reinvent the antibody validation wheel each time an antibody is used. Thus science can build upon itself as it is supposed to do.

Some may argue that one should use monoclonal antibodies to eliminate variability. This is unnecessary and also unwise. It is unnecessary because for most antibodies a single rabbit can produce a stable 20–30 year supply of antibody. Only a small percentage of all antibodies sold ever sell more than can be produced by a single rabbit. It is unwise because monoclonals cost at least 3X what polyclonals cost and we are unlikely to see a time in the near future when cost will be irrelevant. Moreover, polyclonal antibodies have been shown to be superior to monoclonal antibodies in a number of different applications⁴.

Scientists and journals can fix the validation problem if antibody manufacturers will first fix the variability problem. This is called the Antibody Two-Step Solution.

Competing interests

MDB is majority owner and CEO of PhosphoSolutions LLC, an antibody manufacturer.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Angus C. Nairn

Department of Psychiatry, Yale School of Medicine, New Haven, CT, USA

This short commentary article presents an opinion on the ongoing discussion of quality control of commercial antibodies. This is an important topic given the importance of these reagents to progress in a wide range of biomedicine, and of the large economic costs involved in their successful use. But more importantly of the costs when they fail, are subject to variable success, or when they introduce error and artifact.

The comments come from the CEO of PhosphoSolutions, one of many companies who provide and generate general and state-specific antibodies to a wide variety of proteins and epitopes, and who have a vested interest in providing a high quality product. Two general issues are discussed, namely antibody variability and antibody validation. These are closely interconnected aspects of quality control, and two reasonable solutions are presented. In terms of variability, which arises for a number of different reasons, including batch-to-batch differences in a single source reagent, to multiple variants of protein or epitope-specific reagents generated by different individuals or companies, the suggestion to provide a catalog number and supplier is a reasonable start. These sorts of detail should be a minimal requirement, and have been discussed in other recent initiatives (see for example Helsby et al cited; Bandrowski et al <http://dx.doi.org/10.12688/f1000research.6555.1>).

But why not be more specific and provide full details of the characterization of the antigen, epitope if known, and precise lineage related to the origins of the reagent. Importantly, there needs to be specific information that indicates which particular batch is being sold. One could even consider some sort of unique bar code that would enable investigators to keep track electronically of the reagent they receive. Greater transparency and greater detail would benefit all parties involved.

In terms of variability, the reasonable suggestion is made to pool batches to avoid batch to batch variation. The context of the suggestion is that the discussion here is most relevant to the generation of rabbit polyclonal antisera and it makes sense. But in logistical terms, typically initial studies of a particular rabbit serum is done on a pilot basis to establish quality, and antibodies are

often affinity purified in batches. Decision points may vary depending on whether the best strategy is to pool sera, or pool batches of purified antibody. A key missing element is the establishment of robust criteria to establish a quantitative threshold of an acceptable quality that will consistently ensure success of whatever pools of serum or purified antibody are used eventually in the desired application, be it immunoblotting, immunohistochemistry or ELISA.

A third short ending deals with the strengths and weaknesses of monoclonal versus polyclonal antibodies. This is really a different issue from the rest of the discussion of validation and variability which is most relevant to rabbit polyclonal antiserum/antibodies. I suggest that the material in the last paragraph be used as part of a slightly revised introduction and used to set up the context for the later discussion. Monoclonal antibodies clearly have the capacity to overcome the variability issue and are more routine to validate once an assay is standardized. The second sentence "This is unnecessary and unwise" would be best removed, and the strengths of polyclonal antibodies (price and amount per rabbit, superior use in different applications) still made.

Another suggestion would be to remove the ambiguous comparison that "validation is the hardest nut to crack" while also saying "the variability problem" is most important. Better to say that variability and validation are two inter-connected issues, both of which need to have improved quality control.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 29 September 2015

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Andrew D. Chalmers

Department of Biology and Biochemistry, University of Bath, Bath, UK

The opinion piece by Mike Browning makes a clear proposal to reduce the problems associated with using research antibodies. He proposes a two-step process, where step 1 is to reduce variability and the second step is to improve validation. The main contribution seems to me to be highlighting the importance of variability- step 1.

Overall it is nicely written, concise and makes a very useful contribution to the ongoing discussion about how to reduce the problems with research antibodies, something which is crucial to support progress and reproducibility in life sciences. I feel it is suitable for indexation.

I had a couple of comments/questions about the commentary:

1. A reasonable conclusion from the argument about variability seems to me that we should be working towards encouraging researchers to record and report lot numbers of the antibodies they use. Do you feel this would be beneficial?
2. The suggestion of pooling positive serum seems very sensible. I would be interested to know how frequently is this done and if it is not done very often why is this?

Competing Interests: Andrew Chalmers is the founder and a shareholder in CiteAb limited which lists antibodies, including those produced by the author's company.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reader Comment 16 Oct 2015

Mike Browning, PhosphoSolutions LLC, USA

Many thanks for your comments Dr. Chalmers. You raise the issue of lot numbers and suggest that these numbers should also be recorded and reported. As I indicated in my comment to Dr. Valenzuela, lot numbers can be both a blessing and a curse. Unfortunately, the use of lot numbers sometimes masks a tendency among suppliers to fail to follow best practices that will eliminate most antibody variability. The pooling of serum is the first best practice that must be followed to eliminate variability. In my experience few suppliers or researchers do this. See for example Dr. Nairn's discussion of serum pooling in his comment and also [Vivien Marx's article in Nature Methods \(2013\)](#). This is due primarily to an increase in time and effort needed for pooling serum and also possibly to ignorance of best practices among some suppliers. The increase in time is due to the fact that either the research use or sale of the antibody must be delayed until all bleeds of the rabbits have been collected. The increase in effort is due to the need to screen all bleeds to determine which bleeds should be pooled. Many suppliers and researchers start using an antibody as soon as they get a positive bleed. Then when that bleed is exhausted they switch to the next etc. However there can be huge differences in the quality of the antibody in subsequent bleeds. Serum pooling must be used to avoid this form of lot-to-lot variability.

There is a second even more serious form of antibody variability that is often obscured by simply changing the lot number. This results when an antibody supplier runs out of all the bleeds from the original animal(s). Some antibody suppliers then use new animals to make the antibody and simply denote this fact by changing the lot number. The potential differences between bleeds from the same animal pale beside the differences seen in different animals. It is not enough to simply change the lot number. The best practice is to modify the catalog number to reflect the new animal source.

Competing Interests: I am CEO and owner of the antibody manufacturer PhosphoSolutions.

Reviewer Report 25 September 2015

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C. Fernando Valenzuela

Department of Neurosciences, School of Medicine, UNM Health Sciences Center, University of New Mexico, Albuquerque, NM, USA

The paper of Browning proposes a two-step solution to solve problems related to antibody performance in biomedical research. The author proposes that antibody variability must be fixed first (for instance, by pooling positive serum from multiple animals). Once this issue has been addressed, validation will become more straightforward and will progress with time. This well-written opinion article is timely and addresses an important issue that has been the focus of intense discussion in recent years. There are only a few issues for the author's consideration:

1. Although it is clear what the author means by the two-step solution, relatively little detail is given regarding the issue antibody validation. A more detailed discussion of this issue is warranted. For instance, what is the author's take on the approach proposed by [Bordeaux et al.](#) (Biotechniques. 2010 Mar;48(3):197-209. doi: 10.2144/000113382). It is stated that antibody validation will essentially be accomplished as more and more work is done with the antibody. However, there are many factors that limit the access of this information to both suppliers and users. If the antibody does not work on a particular application, the researcher often discards it without reporting any problems in any form. Perhaps being more aggressive about requesting feedback and making sure that all validation information is included in technical data sheets could contribute to solve the problem; the quality of the information included in some web sites and technical sheets is not particularly impressive. Encouraging users to submit information to sites such as [antibodypedia.com](#) or [histoneantibodies.com](#) could also be a step forward. Several companies provide trial size antibodies that allow the users to test them in a particular application in exchange for feedback.
2. What are the author's thoughts on how the task of antibody validation be distributed between vendors and users?
3. Regarding reporting of antibody use, in addition to reporting catalog numbers, it seems as if it would be crucial to report lot numbers as well.
4. What is the author's take on the proposal that an independent body be recruited (e.g., National Institute of Standards) to oversee an antibody certification program?
5. The abstract does not explicitly define the two-step solution.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 12 Oct 2015

Mike Browning, PhosphoSolutions LLC, USA

Many thanks for your comments Dr. Valenzuela. I share your frustration with the issue of antibody validation. However, if variability is not dealt with first, validation has little value to subsequent studies. Moreover the issue is very complex and beyond the scope of this opinion article. I have discussed my perspectives of this issue in my blog. You offer a number of suggestions about validation that I strongly support. These include the proposal you cite by Bordeaux *et al.*, the need for publishing negative data and contribution to sites that catalog validation data.

One issue you raise that I'd like to expand on is the use of lot numbers. Lot numbers can be both a blessing and a curse. Unfortunately, the use of lot numbers sometimes masks a tendency among suppliers to fail to follow best practices that will eliminate most antibody variability. The pooling of serum is the first best practice that must be followed to eliminate variability. Unfortunately very few suppliers do this. This is due primarily to an increase in cost and effort needed for pooling serum and also possibly to ignorance of best practices among some suppliers. The increase in cost is due to the fact that sales of the antibody must be delayed until all bleeds of the rabbits have been collected. The increase in effort is due to the need to screen all bleeds to determine which bleeds should be pooled. Many suppliers start selling an antibody as soon as they get a positive bleed. Then when that bleed is exhausted they switch to the next etc. However there can be huge differences in the quality of the antibody in subsequent bleeds. Serum pooling must be used to avoid this form of lot to lot variability.

There is a second even more serious form of antibody variability that is often obscured by simply changing the lot number. This results when an antibody supplier runs out of all the bleeds from the original animal. Some antibody suppliers then use new animals to make the antibody and simply denote this fact by changing the lot number. The potential differences between bleeds from the same animal pale beside the differences seen in different animals. It is not enough to simply change the lot number. The best practice is to modify the catalog number to reflect to new animal source.

Lastly, with regard to antibody certification by some independent body we wholeheartedly support such efforts to develop a set of best practices. One key issue in such an effort would need to be communication between all the stakeholders including researchers, the journals AND the suppliers.

Competing Interests: I am CEO and majority owner of PhosphoSolutions LLC a company that manufactures and sells antibodies

Reader Comment 13 Oct 2015

Fernando Valenzuela,

I have no further comments. I recommend final approval of this commentary.

Competing Interests: None

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