The Antibody Society presents:

Antibody Validation Webinar Series

WEBINAR 2: Antibodies drive irreproducibility

Moderator: Dr. Simon Goodman, The Antibody Society

Speakers: Professor Glenn Begley, Biocurate Pty Ltd, and Professor Cecilia Williams, KTH Royal Institute of Technology

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Questions and Answers from the live Webcast on November 13, 2019

Question	Answer
According to you, why are there so many bad antibodies on the market?	I think you need to view the whole series to get an overview answer to this complex question: in short, it's because of the lack of appropriate validation. If no-one (we the users) tells the providers and the community that an antibody is "bad", they will remain on the market. So it is part of our job to police the validity of the reagents we obtain. The other sides we shall hear about during the series.
What kind of antigens/proteins are the most difficult to find an efficient antibody for?	Misquoting Animal Farm: "All antigens /proteins are most difficult, excepting some are more difficult than others". It is the correct and fit-for purpose validation that is difficult. The antibody is in effect only "bad" in as far as you do not prove in your experimental context that it is fit-for-purpose.
For a western blot application, what is the best way to validate an antibody for its specificity?	We'll be dealing with that topic in great detail when Prof. Gomes speaks in Webinar #6.
Would you agree that a peptide polyclonal antibody would be specific and selective if the sequence of the immunizing peptide was checked for uniqueness?	Yes, an anti-peptide polyclonal antibody could be specific, but it needs to be confirmed that is the case - it is dangerous to assume it is specific without confirming that is the case. Plus, you have the issue of mimetopes, where unrelated peptides may also fit with high affinity into the antibody binding sites. This will also be discussed later in the series.
Thanks a lot for your error seeking !! I am wondering why the papers that could not have been reproduced have not been retracted.	Like you, I am concerned that the papers the authors could not reproduce have not been retracted. I believe the real problem is that there is no incentive for scientists to correct the record. We need to have some consequences within the scientific system for bad behaviours, but there are very few at this time.

According you, what should be done by the scientific community to solve the antibody specificity problem?	As I mentioned, I believe we need consequences for 'bad behaviours'. Antibodies are important, but they are only one manifestation of the problem of perverse incentives - sadly there is little reward for reliable, reproducible data. The "reward" is for a paper in a top-tier journal which then allows me to get my next grant, get promoted. The rewards are for quantity, not quality. But there is really no incentive within the system for us to perform high-quality science. If we focused on quality, then the sales of poor-quality antibodies would be impacted!
Could you please elaborate on how you would validate a new antibody for IHC? For example, number of tissue samples, types of tissues, specificity, sensitivity?	It is important to have clear positive and negative controls, and ideally from the same tissue type, which is not always that easy. A start is to look for samples with high and low/ zero mRNA levels (if they exist for the protein of interest), and use those as controls. The more controls the better.
Do you think that the manufacturers and suppliers are aware of the lack of good antibodies on the market? Why do they not provide better quality antibody reagents?	I think that many Manufacturers and suppliers fail to validate their antibodies sufficiently - they leave that to the buyer. After all, it is the scientist whose reputation is damaged by a poor-quality antibody that is used to make a claim that is not truly correct: the scientist cannot blame the tool they use, they are as responsible for the tool they choose to use as they are for the result.
Do you think the onus of antibody validation falls onto the vendors or the individual researcher?	It certainly rests on both sides. The user has to make sure the antibody is fit for purpose in their particular experiments (e.g., with appropriate positive and negative controls always in place). On the other hand, the vendor should not be selling anonymous clear liquids, but characterized reagents. Follow the series for very much more on this topic.
The emphasis on QC by individual users is of course essential. Is there consideration for consolidation of such validation (or lack thereof) in a database accessible to all user?	There are several such efforts available (e.g., Antibodypedia, and others). We will be discussing these issues in the next two webinars.
Do you think that researchers should get the amino acid sequence of their useful antibodies and then recombinantly express it in house, to secure their antibody forever?	If at all possible: always get the sequence to immortalize the reagent. One sleeps easier afterwards. Plus you never know what format you may want to put it into in future and the sequence enables that. Regarding the amino acid sequence of antibodies: yes, we would be much better off if we defined antibodies based on their amino acid sequence. That is, of course, how therapeutic antibodies are defined and then approved for clinical use. It would be a real improvement if we applied the same level of rigor to the reagents we use in the clinic as we do in the lab.

Given the challenges we face, and that we are all scientists, what do YOU think needs to happen (question addressed to the viewers)?	
I think that deriving the AA sequence after identifying your key antibody needs to become a necessary stage in the drug discovery process. Given that I don't foresee vendors investing the necessary resources right away to validate their own antibodies, I think we should take this step to safeguard our own research.	Agreed. But as we saw in the first webcast, even monoclonals can be heterogeneous. So deriving sequence directly from protein can be tricky. Even with refined MS tools. But it seems likely that it may be economically viable soon. Maybe there are other options emerging.
The Scientific Community should maybe establish a task force that goes around the labs and checks the reproducibility. This new, feared task force then has the authority to retract a paper if the results cannot be reproduced.	The Affinity-police? I love the idea, but think of the economics... recall there are some millions of available commercial antibodies. I think one option would be much more self reporting in an organized structure. At least some providers will withdraw antibodies reported as "bad" by users. But many, many don't.
Lobby the key funding agencies to encourage high profile journals to require validation (new or by reference) of Antibody tool reagents used in work submitted for publication. The increased confidence should enhance the reputation of the journal and drive similar requirements at other journals over time.	