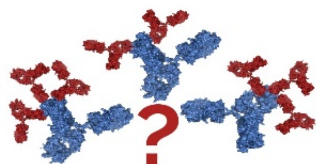

WEBINAR 1: Antibody specificity? What's the problem

Moderator: Dr. Simon Goodman, The Antibody Society

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Questions and Answers from the live Webcast on November 12, 2019

Question	Answer
What is the recommended way to evaluate hybridoma-derived antibodies for true monoclonality?	This is very hard at the protein level. It has to be done by PCR. Mass spec might be an option, but this requires that you know part of the sequence of one of the antibodies.
I would be careful about saying that most experts agree that recombinant antibodies are the future. I would only agree with this if we include hybridoma antibodies that have been sequenced and are made recombinantly. There are many new proteomic and high-throughput B-cell sequencing methods that will bring naturally made antibodies to the fore again.	Recombinant antibodies are to be understood as molecules whose sequence is known. There are many ways as to exactly how this is achieved, one of them being that they originally came from hybridomas. But of course, they can also be obtained directly, without hybridomas.
How would you classify antigens on intracellular proteins in fixed cells? Would they be linear or conformational?	Fixed cells and tissues are special, in the sense that they are crosslinked. They will have some linear and some conformational epitopes. They will be at least partially denatured, by being heated in the "antigen retrieval" step.
1) What do you think about an appropriate method to find RTK, GPCR, or Immuno-oncology antibodies using Hybridoma technology, Display, or Transgenic mouse? 2) Is it possible to find anti-GPCR positive hits using synthetic scFv or Fab libraries?	1) Success has been obtained with each of these methods. GPCRs is by far the hardest among them, so display methods offer the most options. 2) Yes, one can get anti-GPCR antibodies from display libraries, this has been done. The key to success is to create a stable GPCR.
So, should we sequence all the antibodies (reagents) we use in our assays?	Eventually, this is what science needs to move towards. Of course, for the individual researcher this is an undue burden, and manufacturers or repositories will have to have an important role in this.
Can an antibody reactive in immunohistochemistry be used for an in-vivo application to target the same antigen in live cell for therapeutic blockade?	Unlikely. In vivo applications need native antigens and antibodies which recognize those. The requirements for in vivo applications are way higher, of course.
What would be your advice to generate antibodies suitable for IHC? Do you think it is better to immunize with unfolded proteins, peptides, or other types of antigens?	This is indeed a difficult question, and since they are derived from native proteins, and "just" crosslinked and somewhat denatured, starting with the native protein may be a start. Importantly, check them early for the real surface which you want to work with.

So, it does not make sense to analyze an antibody that is intended for therapy with a conventional IHC? Maybe at least IHC with frozen tissue instead ?	Your mileage may vary. In some cases, "antigen retrieval" (after all, microwaving your protein (!)) may destroy your epitopes, in some other cases, it may not.
You stated earlier that in IHC the epitope may be obscured and the antibody may not recognize the target, but is this the same as when the tissue is frozen or paraffin embedded?	Cryosections are much gentler on the protein, and this should be tried if the other methods don't work.