ANTIBODY BOOTCAMP — RISING TO THE FITNESS CHALLENGE

Scientists and suppliers are EMBRACING STRATEGIES to improve the performance of research antibodies and tackle the reproducibility crisis.

Antibodies are invaluable tools in the life sciences. Their high specificity and selectivity for unique protein targets make them indispensable research reagents. Scientists worldwide spend nearly US$2.5 billion a year on antibodies to detect and quantify the expression of proteins in cells and tissues[1].

However, lately, the quality of these reagents has come under intense scrutiny. Not all of them seem to be as selective and specific as was assumed, leading to incorrect, inconsistent and irreproducible results[2]. Alarm bells sounded in 2012 when independent laboratories were unable to replicate the results of 47 out of 53 landmark cancer research papers[3].

"The field has been hampered by antibodies that recognize the wrong (or multiple) protein isoforms and antibodies that don't work well in particular applications," says Andrew Waters, a postdoctoral researcher at the University of North Carolina, Chapel Hill. Waters's own dissertation work was significantly delayed because of an antibody that recognized a nonspecific protein of the same molecular weight as his target protein.

Antibody underperformance can significantly drain research time and money. Months, sometimes years, can be spent trying to replicate experiments or proceed with work that is based on incorrect conclusions. To address this growing problem, researchers need to be aware of the issues surrounding these reagents — and antibody manufacturers need to set higher quality standards.

Common issues and how to avoid them

Although antibodies are designed to recognize a target protein, they may not be able to do so in all applications — namely, those that alter the target protein's structure. Thus, antibodies should be verified in the application of interest.

Antibody performance can also be hampered by binding to off-target proteins when the target is expressed at low levels or has many isoforms. These potential obstacles can be assessed by using appropriate positive and negative controls prior to carrying out the experiment.

Different batches of antibody can produce dramatically different results. Because antibodies are often referred to simply by brand name, it is important to check the manufacturer's lot number and characterization data. This information is often omitted in published articles, making it very hard to track down the actual antibody that was used — and reproduce the findings.

Lack of training in the use of research antibodies compounds these risks. "Many young scientists fail to appreciate the need to confirm that their antibody works in their set-up," says Giovanna Roncador, head of the Monoclonal Antibody Unit at Centro Nacional de Investigaciones Oncológicas in Madrid.

With colleagues from the European Monoclonal Antibodies Network (EuroMabNet), Roncador has produced a comprehensive set of guidelines to avoid common pitfalls in research antibody use[4]. Their recommendations include: defining the target antigen and the experimental techniques that will be used to identify it; conducting a thorough search of the literature to find information on existing antibodies; assessing the available validation data and determining what further validation measures are required; and providing all the necessary protocol and antibody details so others can reproduce the findings.

"OUR AIM IS TO BUILD TRUST WITH THE SCIENTIFIC COMMUNITY AND HELP ADVANCE THEIR RESEARCH."

Other organizations are helping with training: societies such as ISAC (International Society for the Advancement of Cytometry) and ICCS (International Clinical Cytometry Society) are producing webinars and educational materials to help junior scientists select and handle research antibodies.

However, determining an antibody's sensitivity, specificity and reproducibility in a given application — across experiments and over time — is a complex and costly process that researchers can't do on their own. Experts from industry and academia have come together to develop standard guidelines for antibody validation.

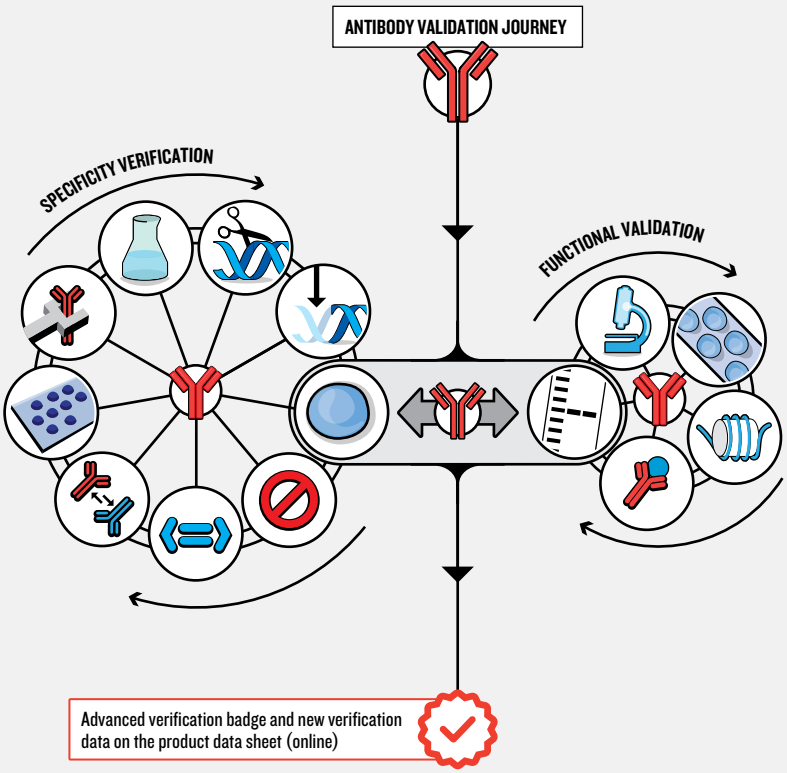
Establishing validation standards

The International Working Group for Antibody Validation (IWGAV) is a consortium of leading protein scientists formed in 2015, and supported by the global life sciences company Thermo Fisher Scientific. The IWGAV has proposed five approaches for antibody validation: using genetics; using an orthogonal (non-antibody) strategy; using independent antibodies binding to the same target; correlating antibody labelling with the expression of tagged proteins; and immunoprecipitation followed by mass spectrometry[5]. At least one of these strategies should be used when validating an antibody for a specific application. Thermo Fisher has used these recommendations as the basis for its own internal systematic approach for verifying the specificity and functionality of antibodies created for its Invitrogen brand (see 'Two-part approach for antibody verification').

Deepa Shankar, director for research and development

TWO-PART APPROACH FOR ANTIBODY VERIFICATION

Rigorous antibody validation is achieved by testing that the antibody binds to the right target in the application of interest. This involves using at least one of nine specificity tests in the applications shown below.



SPECIFICITY VERIFICATION

- Knockout—expression testing using CRISPR-Cas9 cell models
- Knockdown—using RNAi to reduce the expression of genes of interest
- Cell treatment—modulating cell signalling pathways to detect an enrichment, depletion or translocation of the antibody target
- Neutralization—functional blocking of protein activity by antibody binding
- Relative expression—using naturally occurring variable expression to confirm specificity
- IAV—independent antibody validation using two differentially raised antibodies against the same target
- Peptide array—using arrays to test reactivity against known protein modifications
- Orthogonal method—correlation using both antibody-dependent and -independent methods
- IP-MS—immunoprecipitation (IP) followed by mass spectrometry (MS) to identify antibody targets

FUNCTIONAL VALIDATION

- Western blotting
- Immunofluorescent imaging
- Flow cytometry
- Chromatin immunoprecipitation (ChIP)
- Immunohistochemistry

at Thermo Fisher, explains: "We want to help researchers make an informed choice by producing the most compelling data showing that an antibody works." Her team is devoted to validating the company's large antibody portfolio — testing them using Thermo Fisher's two-part approach. "We spend a lot of time ensuring that we test our antibodies in the right environment, in multiple models and in different applications," she says. "Our aim is to build trust with the scientific community and help advance their research."

Detailed testing protocols and results, as well as published antibody data, are collated on the company's website. "Customer feedback is really positive," says Shankar. "We are seeing a growing number of publications using our antibodies demonstrating that they are working."

In recognition of these efforts, Thermo Fisher won the 2018 CiteAb Award for the best antibody validation initiative. "Rigorous validation procedures are not in place in many laboratories. Lack of awareness, resources and funds means researchers are relying on vendors to provide good antibodies," explains Paul Wallace, director of the Department of Flow & Image Cytometry, Roswell Park Comprehensive Cancer Center in Buffalo, New York, and a panel member on Thermo Fisher's Antibody Validation Forum. "I am very impressed by how Thermo Fisher is taking responsibility for the quality of its antibody products — and is open to dialogue with users."

Bright outlook

The first step in solving any problem is to recognize that it exists. Since the issue of antibody validation was exposed, it has been openly discussed — and many initiatives set up to find the best solutions. "We are making headway, but a lot more still needs to be done to figure out what are the best strategies to address the problem," says Wallace. Agreeing to the need for antibody validation standards is a significant first step. Given the importance of reproducibility for the advancement of science, it is in the interest of all researchers and suppliers to step up to the challenge of implementing these standards. ■

REFERENCES

1. BCC Research. *Global Markets for Research Antibodies* (2018).
2. Baker, M. *Nature.* **521**, 274–276 (2015).
3. Begley, C. G. & Ellis, L. M. *Nature.* **483**, 531–533 (2012).
4. Roncador, G. *et al. mAbs* **8**, 27–36 (2016).
5. Uhlen, M. *et al. Nature Methods.* **13**, 823–827 (2016).

ThermoFisher SCIENTIFIC