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Editorial

Recommendations for description and validation of antibodies for research use

1. The importance of antibodies in research

A plethora of experimental methods rely on specific antibodies, such as Western blotting, immunohistochemistry (IHC), chromatin immunoprecipitation, flow cytometry, enzyme-linked immunoassay, antibody arrays, electrophoretic mobility shift assays, and mass-spectrometry-based immunoprecipitation proteomics, among others. However, the antibodies available on the market are often relatively non-specific and can show enormous batch-to-batch variations. The possible exceptions of this variability are antibodies that are approved by the FDA or other equivalent national health regulatory bodies as diagnostic tools or therapeutics [1–4]. At present, vendors provide different levels of antibody validation, and they are not required to perform quality control for every new batch [5]. Together with frequent underestimation of the level of complexity of immunoassays, this can all lead to erroneous data, and even retraction of published papers. In the last decade, the scientific community has recognized this serious problem, which has led to the development of a set of publication guidelines for appropriate descriptions of experimental data obtained with antibodies [6–9]. There is a growing awareness among scientists that the use of imperfect tools can result in incorrect interpretations of experimental data [10]. Now it is a high time that journals, as the gatekeepers of information, take a lead and enforce full and correct descriptions [11] that also contain validation data on antibodies that are used. Several journals currently request data on antibody specificity and validation within the text or in the form of a table [6,12,13], and the Journal of Steroid Biochemistry and Molecular Biology is now joining this initiative by providing clear and comprehensive instructions for authors.

2. Requirements for antibody information

Essential information thus includes detailed data not only on the antibody itself and its validation, but also on the pre-analytical, analytical, and post-analytical processes that can also affect the final results [14,15]. For instance, when performing immunohistochemistry (IHC), the fixation, antigen retrieval methods, and tissue processing can have strong effects on antigenicity by inducing conformational changes in the epitopes, limiting their accessibility, or generating high non-specific background [16].

2.1. Description of an antibody

Based on the initiatives from scientists from academia and industry, the description of an antibody should ideally include the following [7,11,12,15–17]: (i) unique and complete information on the antibody (i.e., source of the antibody, antiserum code number, vendor, catalogue number, lot number); (ii) information on the preparation of the antibody (i.e., specific properties of the antigen, species used to raise the antibody).

2.2. Validation of an antibody

For the validation of an antibody, ideally several of the following techniques should be used:

1) Western blotting with tissue samples and cell lines where the antibody should stain a single band (or several bands if there are different isoforms or post-translational modifications). Here scientists should be cautious, and should not rely on the quality control data provided in the technical information from the manufacturer, as these can correspond to a different lot.
2) The specificity of an antibody can also be validated by:
   (i) comparison of IHC staining with the antibody with the staining pattern previously reported for another antibody validated against the same protein;
   (ii) comparison of the staining and co-localization with two antibodies raised against different epitopes of the investigated protein, or raised in different species;
   (iii) comparison with mRNA levels by qPCR or *in-situ* hybridization;
   (iv) comparison with profiles from quantitative protein arrays (e.g., the human protein atlas [18]).
3) There are also other emerging technologies that can be used for antibody validation, such as proximity ligation assays [4,19], while optimally, the specificity of an antibody should be confirmed by mass spectrometry after immunoprecipitation experiments [20].

2.3. Positive and negative controls

The appropriate positive and negative controls should comprise: (i) tissue of the wild-type and knock-out animals;

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Table 1
Required description of antibodies and their validation.

Information on the antibody					
Name of the antibody	Manufacturer, catalogue #. batch #, name of the source	Peptide/ protein target	Antigen sequence	Species raised, monoclonal, polyclonal, antigen purified	Dilution used (mass concentration if affinity purified), reagents
Antibody validation					
Western blotting	IHC staining	IHC staining		Other methods	
Positive/ negative controls	Positive/ negative controls	IHC staining pattern as reported previously for another antibody (provide citation)	IHC with two different antibodies against the protein (include information about the antibodies)	Comparison with mRNA levels by qPCR and *in-situ* hybridisation patterns (experimental data or citation)	Comparison with targeted proteomics profile (experimental data or citation)

The table format is recommended, although the same information can be provided as text.

(ii) transfected cell line and/or cell line where expression of the investigated gene/protein has been silenced (i.e., siRNAs, shRNAs); and (iii) tissues or cell lines that have previously been unequivocally shown to express (or not to express) the investigated gene/protein. An absorption control should be used with caution, as it does not prove specificity of an antibody; pre-adsorption with the blocking peptide can prevent binding to the target as well as to off-target proteins [13,15,21].

2.4. Suggested reporting format

The required data will best be presented in table format (Table 1), with additional information explained in the accompanying text.

2.4.1. Western blotting

Provide data on sample preparation, quantity of samples loaded onto the SDS PAGE gel, conditions of transfer to the membrane, reagents used to reduce non-specific binding, duration and temperature of incubation with blocking solution, duration and temperature of incubation with primary and secondary antibodies, types and concentrations of secondary antibodies, detection reagents, quantitation of Western blotting (if applicable). Provide figure showing the whole membrane with molecular weight markers, and with negative and positive controls [4,7,16,17,22].

2.4.2. Immunohistochemistry

Provide data on tissue handling, fixation and processing (*e.g.*, type of fixative, fixation procedure, reagents, concentrations, duration, temperature), storage and handling, antigen retrieval (if applicable), IHC detection methods and staining procedures (*e.g.*, primary and secondary antibodies, concentrations, reagents used to prevent non-specific binding, duration, temperature), detection system and reagents, description of the control experiment, manual or automated staining platforms and software (if using automatic immunostaining, the details of the instrument including the manufacturer, model number, and programme used in the staining), manual or automatic data interpretation (software used). Provide figures showing IHC with positive and negative controls, primary antibody control, secondary antibody control, and staining controls [4,7,16,17,22].

When using surgical pathology or archival data, type of fixation, approximate duration of fixation and type of surgery materials should be provided. Although tissue microarrays (TMAs) have essential roles in antibody validation [4,8,14,15], the analysis of TMAs in neoplasms is by no means ideal, and has provided erroneous information for results or interpretation due to intratumoral heterogeneity, so should be discouraged when whole section specimens are available. However, when applying IHC in TMA specimens, the following information must definitely be provided:

1. How many cores were obtained from each specimen?
2. From which areas were the cores obtained, such as the centre of the tumour or the invasive front, or other areas?
3. The correlation of IHC findings between those in TMA and the same whole specimens must be performed in at least 10% of the cases examined, and the results should be statistically significant.

3. Closing remarks

This editorial tackles part of an immense current problem in science, the irreproducibility of data [23]. The suggested recommendations include detailed reporting of technical procedures and meticulous validation of antibodies following suggested algorithms [4,15]. These will thus decrease the enormous time and resources scientists can waste on inappropriate antibodies, and most importantly, it will help to prevent publication of erroneous data, with the principal aim to increase the quality of published research.

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