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Review Article

Quality Issues Of Research Antibodies.

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Abstract

A surprising percentage of seminal articles appear to be unreproducible by independent labs, per a number of recent investigations. An important part of this is played by nontherapeutic antibodies used for environmental, dietary, diagnostic, research, and other applications. issue. Even though several articles have been published with recommendations for how to make things better, they don't seem to be thorough enough to address the intricacy of this problem in its entirety. Furthermore, there haven't been any discernible advancements in the subject thus far. This article attempts to create a more cohesive idea by combining the astounding range of findings and recommended actions.
It is determined that in order to address these issues and pave the path for a more sustainable approach to bioanalytical research that everyone can trust, funding organizations and journal publishers must act quickly.

Keywords : *reproducibility crisis, irreproducibility, replication, paper retraction, peer review, immunochemistry, immunoassay, ligand binding assay, monoclonal antibody, quality control, cross-reactivity, non-specific binding, selectivity.*

INTRODUCTION

Scientific studies' reproducibility has been questioned on numerous occasions. 1–8 The majority of the reviewed seminal articles in their field could not be replicated by multinational corporations like Amgen and Bayer HealthCare. 9, 10 Biological reagents account for by far the greatest portion of the research expenditures that are allegedly squandered in the USA, at over 36%. This represents losses of roughly $10 billion USD annually. It is concerning because there appears to be no relationship between a scientific study's repeatability and the impact factor or citation count of the relevant journal. The role of antibodies is important in many of the bad cases that have been described. Even if the initial conversations began at least 20 years ago

There haven't been any significant developments as a result of the attempts to improve the situation. A recent spate of papers addressing antibody quality control (QC) demonstrated the issue's ongoing relevance and attention. 14–16 According to a recent study17, over 300 businesses provide over 2 million antibodies for research. A big bioinformatics business independently examined over 6,000 commercial antibodies from 26 suppliers, according to a remark on this paper. Over 75% of these antibodies were either nonspecific or completely ineffective. In addition, the Human Protein Atlas consortium analyzed over 5,000 commercial antibodies, of which over half were unsuitable for the intended use.

This article attempts to provide a methodical examination of the issues at hand and attempts to compile the majority of the comments and recommendations into a more thorough and ranked list of suggested solutions. This could result in a more sustainable model of research antibody creation and production as well as a significant improvement in the caliber of studies and applications carried out with antibody reagents.

THE STATUS QUO

Development and production of antibodies

This article will discuss antibodies from a variety of sources, including recombinant antibodies, monoclonal antibodies made using Köhler and Milstein's hybridoma method, and polyclonal antibodies derived from blood serum. It should go without saying that an antibody manufacturer or reseller should provide at least the most basic information regarding the antibody production process. Thankfully, most of the

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time, this is accomplished. A unique issue arises rather regularly when it comes to hapten or peptide antibodies. Many businesses claim that this information is proprietary, even though the manufacturer should have full knowledge of the hapten, linker, immunogen, and carrier protein.

Traceability of antibodies

Antibody identification must be done clearly and unambiguously. Only 44% of the antibodies described in papers can be identified at all, per a recent publication (20). Additionally, there is no correlation between this fraction and the journal's impact factor. The majority of antibody characterization must be done by each user if an antibody cannot be properly identified by a label. Otherwise, the individual using the antibody is unaware of its characteristics. Remarkably, for a large number of publications, this status is almost the norm rather than the exception. Since the corresponding experiments cannot be replicated independently, these studies should not be regarded as scientific in the strictest sense.

Concentration and activity of antibodies

Since storage and transportation conditions have a significant impact on an antibody's aging, this factor is challenging to regulate. On the label or data sheet, the manufacturer often indicates the protein or IgG concentration. This is merely a stand-in for the pertinent data, which is the amount of active antibody. Determining a concentration for polyclonal antibodies is especially challenging because, in this case, even the IgG content is essentially meaningless. Since it is not a given that every antibody included in such a preparation will have the same or any activity in a particular assay or application, antigen-affinity-purified antibodies likewise exhibit this issue. As a result, the reagent's present concentration or activity may not be as high as anticipated.

Affinity constants

Spread your knowledge. Meanwhile, several antibody vendors and other, more independent sites offer the opportunity to rate antibodies. All things considered, this is an excellent method of disseminating crucial information that was previously either extremely limited or nearly unattainable. Unfortunately, the number of ratings and comments is still quite low. Therefore, whether this approach is advancing the field overall is yet unknown.

This is accomplished through the use of ilibrium dialysis and other methods. Affinity constants can be regarded as a stable property that is practically always valid, provided that the measuring conditions are specified. It is unfortunate that affinity constants for antibodies and their corresponding antigens are rarely determined. A perfect characteristic of an antibody-antigen combination, an affinity constant has a remarkable impact on almost every application. Only a very tiny percentage of antibodies with affinity data are now available for purchase.

Cross-reactivities or selectivity

There have been several definitions of antibody cross-reactivities put out, but only one—first published by Abraham—has acquired widespread acceptance. 26, 27 Broad group selectivity (27,33) or 100% selectivity (specificity) 28–32 are preferable in many applications. However, undesirable and even totally unanticipated cross-reactivities—interactions of more or less unrelated compounds—are always possible because of the lock-and-key mechanism of the antibody–antigen interaction. 34 The only practical query is whether or not the corresponding cross-reactivity is pertinent to the particular analytical application.

For example, a synthetic cross-reactant is obviously meaningless in practice because it does not occur in any real samples. 35 The arbitrary selection of the cross-reactants evaluated is one of the main drawbacks of cross-reactivity investigations conducted nowadays. It is clear that only a very small percentage of the billions of proteins or millions of known chemical compounds that could be present in complicated samples can be analyzed independently. Nowadays, a lot of commercial antibodies are offered for sale with either no cross-reactivity data at all or incredibly inadequate data. "Cross-reactivity: 100% Analyte X" is frequently provided as the only piece of information.

But this information is almost never accessible. This could be because such an epitope scan is expensive and complicated. However, an antibody directed against a protein that lacks a defined binding epitope must be regarded as only partially described. The identification of matched antibody pairs that are appropriate for the sandwich immunoassay setup, which is a noncompetitive test, is a related but even more important issue. Two antibodies that can bind two distinct epitopes without causing steric hindrance are required for such a matched pair. Since there are typically no chemically defined (and validated) antigens accessible for immunohistochemical applications, cross-reactivities in the conventional sense cannot be identified.

Western blots may be in a similar situation, where the appearance of a single band with a roughly sufficient molecular weight is sometimes seen as an adequate QC. It is seen to be a good idea to choose known test samples, such as positive and negative controls. However, other methods, such as the evaluation of one or more pathologists, are required to classify cells or tissues into a type or condition. This evaluation may vary greatly in challenging situations, which could lead to ambiguity in the definition of standard samples. Furthermore, it is impossible to ensure long-term availability because standard or reference tissue samples are

only available in small quantities.

A fresh batch of the same tissue may behave differently with certain antibodies because it may have distinct hidden characteristics. RNA interference, which should suppress the corresponding antigen's gene expression, is another method used to test the selectivity of antibodies in biological materials. 37 In particular, small-interfering RNA (siRNA)-based assays are frequently employed for antibody validation. Papers involving knockout animal experiments are usually withdrawn since an antibody shouldn't detect a target that doesn't exist. The purported selectivity of an antibody may not be sufficiently supported by even a limited number of preadsorption assays or inhibitory tests using a peptide antigen. Twelve HPLC-ELISA and LCELISA immunograms may be useful for environmental samples or other samples that are intrinsically complicated and unpredictable.

Application tests

Many antibodies, like ELISA, WB, and IHC, which are the acronyms for certain important immunochemical procedures, at least carry some information. More thorough procedures are only provided in some situations, though, and regrettably, some assay-specific experience is rarely transferable to another, so such an application statement is only marginally relevant. Applications are frequently the sole pertinent details on a commercial antibody's (preparation) availability.

Reference materials and standards

The manufacturer occasionally provides a positive control, which could be an antigen sample. Assay development and activity assays may benefit greatly from this. However, there is frequently a lack of information regarding this positive control as well. Almost every issue that arises with the antibodies also affects the standards. Due primarily to stability and diversity concerns, certified reference materials from institutions like NIST (National Institute of Standards and Technology), BAM (Bundesanstalt für Materialforschung und -prüfung), or others are almost never available.available, mostly as a result of diversity and stability concerns. Obtaining additional reference materials of any caliber could likewise be challenging. It appears unrealistic to anticipate major advancements in the foreseeable future given the almost total deficiency of these materials (compared to the 2 million commercial antibodies). However, for certain test types, RNA interference materials may be included for control purposes.

Long-term availability

The majority of the published antibodies are no longer accessible after a few years. This appears to be an unjustifiable waste of resources and knowledge, given the substantial effort and financial investment made in the development of the corresponding antibodies. Remarkably little attention is paid to the ongoing loss of antibodies with special structure and characteristics that cannot be recovered. Given this, the recent recommendation to choose recombinant antibodies42 makes a lot of sense because DNA synthesis can even reverse the total loss of a clone in the event of a published sequence of a recombinant (or monoclonal) antibody. A small number of clones are kept in long-term depositories, preferably in various aliquots at several sites.

Unfortunately, insufficient risk assessment, cost constraints, and intellectual property concerns frequently result in inadequate measures being adopted, which ultimately leads to the appalling state of affairs today. Dependency on a single antibody source by researchers and regular analytical chemists is a dangerous scenario. Occasionally, commercial suppliers' test kits or polyclonal antibodies are swapped out without warning, which could cause anxiety in the impacted analytical labs when they find an inexplicable divergence. The end consequence might be the purchase of one or more mass spectrometers and the elimination of immunochemical methods. An unpredictable or even stopped antibody supply could have serious repercussions for researchers.

Antibodies as a subject in publications

A publication, which shares the knowledge presented in the corresponding paper, is frequently the end result of research. As a result, the current publication mechanism might have some bearing on the problem of antibody quality. Numerous problems with conventional publication methods have already been noted, raising doubts about the caliber of antibodies reported in these studies as well as the validity of scientific work generally.

As was already noted, a number of requirements must be met in order to adequately verify an antibody. The description, characterization, and validation of research antibodies are almost never adequate, even in highly cited articles and highly respected journals. Furthermore, the production, description, and verification of antibodies are generally disregarded, making them challenging to publish. The ease with which research reporting novel approaches based on unidentified antibodies and irreproducible immunochemical techniques can be published contrasts sharply with this. Given that there are thousands of reputable publications available today, it is astonishing that there isn't a single journal on the market dedicated to antibody creation and validation.

Antibodies as a commercial product

The process of developing antibodies is costly. But in a business setting, a return on investment must occur within a reasonable amount of time. The topic was recently covered in an intriguing work titled "The Antibody Dilemma,"43 which examined the problem from the perspective of a conventional antibody maker. "Antibody haste, research

waste," is the authors' summary. This appears to be the result of both customers viewing antibodies as a convenience product without taking into account any limits and assuming no responsibility, such as for the application of appropriate negative and positive controls, and manufacturers and resellers taking certain quick cuts to market.

It is crucial to distinguish between therapeutic antibodies, which are ultimately marketed in pharmacies, and research antibodies used for experimental diagnostic, environmental, food analytical, or other objectives. Sales of therapeutic antibodies could reach the billions (109) of US dollars. Research antibodies can occasionally not even cover the thousands (104) of US dollars that were spent on their development. This is roughly 105 times lower! As a result, almost any type of characterisation and ongoing, comprehensive quality control of therapeutic antibodies are not difficult. When determining how the antibodies are produced, this distinction is equally crucial.

Generally speaking, a polyclonal antibody can cost around $1,000, a monoclonal antibody around $10,000, and a recombinant antibody, including some more complex affinity maturation, up to $50,000. It is evident why the majority of commercial research antibodies are still polyclonal when these expenses are contrasted with the yearly sales of a research antibody. For a research application, even a monoclonal antibody may never break even. Some businesses, however, have begun to address the problem and are putting their own quality efforts into action. According to one company, following a more comprehensive quality check, around one-third of its catalog was thrown out.

Academic sources

Academic teams have created a large number of primary antibodies, and they frequently attempt to market the antibodies once the project is completed. Given that many academic groups' antibodies are of excellent quality and adequately characterized to be deemed suitable for their intended use, this strategy appears to be sound. However, test kit producers frequently purchase high-quality antibodies because they do not want the same antibody to be widely available or sold in the market. Consequently, there is no commercial supply of these antibodies.

With very little information on their performance, antibody resellers may be given access to all other antibodies (or clones) of questionable quality that cannot be used for a test kit. This process of negative selection reduces the likelihood that a buyer will obtain a high-quality antibody in the open market. The second issue is that since they have already sold the antibody samples or because their Office of Intellectual Property Administration prohibits it, these academic researchers are no longer willing or able to provide any to other researchers. This transfer frequently keeps the antibodies or clones from being placed in any repository (such ATCC, ECACC, DSMZ, or others), which exacerbates the dilemma. This is frequently where the story ends.

This list of issues might not be all-inclusive. It should be recognized that these issues have persisted for decades and are undoubtedly difficult to fix. Some researchers might even contest the existence of any issues at all, arguing that the research proceeded well without any additional steps. The following ideas for an antibody quality initiative can be viewed as a starting point for additional conversations and as a resource for anybody involved in antibody research or antibodies in general. Urgent action is necessary for a number of reasons. First, science as a whole experienced an intolerable reproducibility crisis.

From a strictly economic perspective, rces is unacceptable. Third, patients and other individuals who rely on accurate results from diagnostic and other analytical procedures may suffer if antibodies of questionable quality are used. Fourth, since the irreproducibility of antibody reagents causes evasive reactions, such as the switch to mass spectrometric techniques in clinical laboratories, which hope to obtain more reliable results then, poor antibodies and immunoassays harm the reputation of an entire analytical field and eventually destroy the financial foundation of many businesses. 44–48 Lastly, many scientists find working with antibodies intimidating because of the frustration that poor antibodies can bring.

WHAT COULD BE DONE?

In every facet of antibody work, complete antibody traceability must be ensured. Like the CAS # for chemicals, DOI for papers, or Researcher ID for scientists, a distinct antibody ID ought to be implemented. It is no longer appropriate to utilize antibodies for research purposes without an assigned antibody ID. Nowadays, fewer than half of the antibodies reported in papers can be identified. 20 Recently, an online antibody registry was launched. The same antibody offered by different suppliers may, regrettably, receive several ID numbers because the operators appear to rely on vendor information, leading to unacceptable duplicates and variable data quality.

All publically sponsored research should serve as the foundation for the establishment of an antibody heritage program. A nonprofit organization, like the ATCC, which currently has a collection of 1,200 hybridomas, should deposit all hybridoma clones of monoclonal antibodies that have been published and/or characterized on a specified minimal level as a first step. Nonetheless, there may be a problem with the commercialization of publically supported antibodies. Other researchers that are interested in a clone or antibody may find it more difficult to get and utilize this reagent if commercialization is permitted as it is now and is

not restricted to a nonexclusive licensing model.

Then, it could be necessary to spend tax dollars twice to produce a new antibody. Privately funded antibodies are anticipated to produce more income and profits nevertheless, and they might not exhibit many of the issues outlined in the article's first section. To minimize disappointment or a damaged reputation, a corporation may benefit from making their products more palatable for the users' quality control. There used to be a symbiotic relationship between the manufacturer of an antibody and its user, as explained in the paper by Ascoli and Birabaharan43.

In order to restore knowledge that has been lost over the previous few decades, biochemical, clinical, and biological societies—as well as maybe commercial antibody developers and resellers—should provide training to antibody users. The proper usage of antibodies is just unfamiliar to many researchers. Assuming that an antibody is a biochemical standard reagent, they also fail to recognize the complexity of antibody reagents, which results in the previously described negligence and lack of recognition for the production of high-quality antibodies.

All grant submissions that discuss antibodies in an experimental setting must have antibody quality workflows established by funding organizations. First and foremost, at least one applicant needs to have adequate experience with antibodies. Second, the project's sponsored hybridoma clones ought to be placed in a nonprofit collection as soon as feasible. This must be required. Third, an antibody ID needs to be given to each antibody clone created for these initiatives. Fourth, to prevent needless duplication of effort, uninformed applicants should be directed to already-existing antibodies.

For almost all researchers who get public funding, publications are quite important. As a result, many scientific decisions are ultimately influenced by the requirements of journal editors and referees. Publishers and editors may be able to make some major improvements because of their direct impact on scientific procedures. This strategy may be implemented quickly and would be incredibly cost-effective. Strict guidelines for the reporting of procedures and findings should be established by all journals that publish work involving antibody research. Antibodies are occasionally regarded as confidential information by one of the parties. Because the reagents cannot be replicated independently, the corresponding study cannot be published in scientific publications when they are not disclosed.

The majority of journals regard novelty as the most important acceptance criterion. However, if the experiment cannot be replicated or is not even correctly comprehended, what does novelty mean? The third rule should be that qualified reviewers must evaluate antibody work. The use of questionable reagents in crucial processes can only be identified by scientists with extensive knowledge in the subject. To maintain a constant standard of quality, these parts should ideally be overseen by committed referees. The situation would immediately improve if almost all journals consistently rejected irreproducible antibody work based on undefined, uncharacterized, and undisclosed reagents. Lastly, it is clear that there aren't many journals devoted to the synthesis, description, and validation of antibodies.

As long as the sequence information is preserved and the published sequence is accurate—which cannot be assumed—antibody sequence information provides a potent means of preventing antibody losses42 and ensures the nearly endless availability of an antibody. Given that the sequence of any recombinant antibody is readily available, the recombinant method of producing antibodies is an appealing strategy in this situation. As is evident from any pricing list of recombinant proteins, the recombinant technology has yet to provide an affordable method of producing reagent antibodies. It must be emphasized that long-term antibody identification and security depend on having a strong understanding of a sequence.

Produce your own antibodies. Since the antibodies can be best streamlined for the intended usage, this is not the worst choice. Nevertheless, this is a rather costly method that requires a great deal of time and expertise. One significant benefit is the consistent supply of antibodies, which many resellers are unable to provide. According to Baker's17 study, if access to your old antibody is cut off, serious repercussions may ensue.

Verify every antibody you receive in the lab. The datasheet's claims should not be trusted. 17 A proper test must be performed prior to the usage of any novel antibody in research. This advise is unpopular and will often be disregarded. This is partly because to the well-known pressure from publications. As a result, many scientists believe they must cut corners or just don't have the time for further quality control. All researchers who have worked with antibodies for a longer period of time are aware that this mindset will backfire.

Disseminate your expertise. In the interim, the chance to rate antibodies is provided by a number of antibody suppliers and other, more independent platforms. Generally speaking, this is a fantastic way to share important information that was previously either very limited or practically impossible. Regretfully, there are still very few ratings and comments. As a result, it is currently unknown if this strategy is improving the field as a whole.

CONCLUSIONS

The two most promising steps to achieve an immediate turnaround are for funding agencies to implement antibody quality restrictions, which might be implemented in the near

future. Last but not least, all pertinent publishers ought to establish unambiguous, non-negotiable guidelines for the reporting of antibody experiments.

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