Antibody Validation

Standards, Policies, and Practices

Workshop Report

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DISCLAIMER

This Workshop Report (Report) highlights the main points, key considerations, and recommendations discussed during the Workshop. The opinions, conclusions, and recommendations in this report do not necessarily reflect the views of the sponsors, the Global Biological Standards Institute, the National Institutes of Health, The Antibody Society, and the American Type Culture Collection.

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ABOUT GLOBAL BIOLOGICAL STANDARDS INSTITUTE

The Global Biological Standards Institute (GBSI) a non-profit organization, is dedicated to enhancing the quality of biomedical research by advocating best practices and standards to accelerate the translation of research breakthroughs into life-saving therapies. GBSI was established in 2013 by the American Type Culture Collection (ATCC), through the leadership of Dr. Raymond Cypess, ATCC's CEO, who recognized the need for an independent organization that would provide thought leadership to advance best practices and standards. BioNexus, an ATCC foundation, continues to provide major support for GBSI's mission and core activities as GBSI builds its base of philanthropic, corporate, and membership support.

GBSI engages expert stakeholders and seeks community input from across the biomedical research field to ensure best practices and standards address critical gaps. Through science, education and training, and policy and awareness, GBSI aims to enhance the credibility, reproducibility, and translatability of pre-clinical biomedical research. GBSI's focus on research reproducibility includes the following projects: Reproducibility2020, the Cell Authentication Alliance, Biospecimen Commons, and the Antibody Validation Initiative, of which the *Antibody Validation: Standards, Policies, and Practices* Workshop is a part.

ACRONYMS AND ABBREVIATIONS

ATCC	American Type Culture Collection
CRISPR	Clustered regularly interspaced short palindromic repeats
GBSI	Global Biological Standards Institute
ICC	Immunocytochemistry
IHC	Immunohistochemistry
IWGAV	International Working Group for Antibody Validation
NIH	National Institutes of Health

GLOSSARY OF TERMS

Affinity: Binding strength between the antibody's binding site and its epitope.

Antigen: A substance stimulates the production of and binds to antibodies.

Avidity: Overall binding strength between the antibody and its antigen.

Certification: Formal demonstration of proficiency in a specific body of knowledge or process.

Chromatin Immunoprecipitation: An experimental procedure used to determine whether a particular protein binds to or is localized to a specific genomic DNA sequence *in vivo*.

Cross-reactivity: Ability to bind other antigens containing similar epitopes as the target antigen

Epitope: A part of the antigen to which the antibody directly binds.

Feasibility: The capability of being done or accomplished.

Flow Cytometry: A technology that is used to analyze and quantify properties of single cells, one cell at a time.

Functionality (Utility): Ability of antibody to work in defined experimental applications

Guideline: A general rule, principle, or piece of advice.

Immunocytochemistry: An experimental technique to visualize the localization of specific antigens in cells using antibodies that bind to the antigens of interest.

Immunohistochemistry: An experimental technique to visualize localization of specific antigens in tissues using antibodies that bind to the antigens of interest.

Immunoprecipitation: An experimental technique to isolate and concentrate proteins out of solution using an antibody that specifically binds to the protein of interest.

Mass Spectrometry: An experimental technique used to analyze the composition of molecules by measuring the masses of chemical species within a sample following ionization of the chemicals and sorting of resulting ions based on their mass-to-charge ratios.

Microarray: A technology used to examine thousands of DNA molecules or expressed genes at one time.

Peptide Array: A technology used to examine thousands of protein fragments at one time.

Principle: A fundamental proposition that serves as the foundation for a system of behavior.

Proficiency Testing: Formally assessing an individual's capability for a conducting a specific task or practice.

Quality Assurance: The process of checking whether a product or service has been made or carried out at the desired quality.

Quality Control: The system for verifying and maintaining a desired level of quality or standards in a product or service.

Reliability: The ability for a product or service to be depended on for accuracy, function, and accuracy.

Reverse Phase Protein Array: A high-throughput technology to quantify the amount of antigen in tissue or cell lysates, serum, plasma, or other bodily samples that are immobilized on a solid surface.

Sandwich Assay: An experimental method that detects the presence or measures relative concentration of a molecule through binding of antibodies used for antigen-capture and detection.

Selectivity: Ability to preferably bind the target antigen in the presence of other antigens.

Sensitivity: Extent to which antibody detects small amounts or slight changes in the abundance of the target.

Specificity: Ability to recognize the target antigen.

Standard: A level of quality or achievement that is considered widely acceptable or desirable.

Suitability (of a reagent): The degree to which the product or service can carry out a particular use or job.

Training: The action of teaching a person a particular skill or type of behavior.

Utility (Functionality): Ability of antibody to work in defined experimental applications.

Validation: The process through which the performance of products and services is assessed.

Western Blot: An experimental technique used to detect and analyze specific proteins from cellular extract or solution (as for detection of purified proteins).

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#AbValidate

ABOUT THE ANTIBODY VALIDATION: STRATEGIES, POLICIES, AND PRACTICES WORKSHOP

In recent years, reproducibility of pre-clinical research has been highlighted as a significant challenge to the practice and translation of biomedical sciences. (*Begley & Ellis, 2012; Freedman et al, 2015a; Freedman & Inglese, 2014; Hartshorne & Schachner, 2012*). An estimated $28 billion dollars are spend on irreproducible pre-clinical annually (*Freedman et al, 2015a*). The root causes of irreproducibility are undeniably complex, influenced by commercial interest, financial resources, scientific knowledge, and technical and/or analytic skills.

A major contributor to irreproducibility are poor-quality reagents, especially antibodies, which are among the most commonly used biological reagents in biomedical research. (*Baker, 2015b; Begley & Ellis, 2012; Bradbury & Plueckthun, 2015a; Marx, 2013; Weller, 2016*). Currently, antibodies used in research are not validated in the same manner as those used in clinical settings. (*Bradbury & Plueckthun, 2015a*) However, variability in quality and functionality of research antibodies has set back basic research studies and development of new diagnostic tests. (*Baker, 2015*b) These setbacks clearly demonstrate the need to develop standards for validating research antibodies and sharing information about the quality and consistency of antibodies in the market. Developing these validation standards with key stakeholders, including commercial and academic producers, distributors, and end-users, increases the likelihood that the standards are scientifically informative, feasible, and adoptable by all relevant sectors.

GBSI established its Validation Initiative in 2014 through the creation of the Antibodies Task Force, which included thought leaders in antibody research, development and production, and journal editors and service providers. As part of this initiative, GBSI conducted a survey of over 500 life scientists to identify current approaches for validating antibodies used in pre-clinical research. (*Freedman et al, 2016*) The survey demonstrated that young scientists and trainees lack the awareness and training needed to evaluate and validate antibody reagents. These findings highlighted the critical need for training on the selection, validation, and application of research antibodies and emphasized the need for transparency of information about affinity reagents, their batch histories and quality. Furthermore, the findings revealed opportunities for shaping the creation of antibody validation standards, best practices for validating antibodies, training and proficiency testing, and product or manufacturer certification, which together, begin to address the irreproducibility problem associated with research antibodies.

ANTIBODY VALIDATION STANDARDS: STRATEGY, POLICIES, PRACTICES WORKSHOP

In addition to the Task Force and survey, GBSI joined forces with The Antibody Society to support the stakeholder meeting described in this Report and began developing consensus on standards for research antibody validation. To promote the development of consensus among disparate stakeholder communities (i.e., researchers, producers, distributors, journal editors, funders, and moderators of antibody databases), GBSI embarked on a three-part strategy involving pre-Workshop online dialogues, the Workshop itself, and post-Workshop Working Groups. The culmination of these efforts will be the development of consensus antibody validation standards. During the planning phase of the Workshop, the International Working Group on Antibody Validation, chaired by Dr. Mathias Uhlén, published a commentary in *Nature Methods* describing a high-level approach to antibody validation based on pillars or foundational strategies to assess antibodies for use in different experimental applications (Uhlen et al, 2016). The key points from this paper were discussed in the pre-Workshop dialogues and at the Workshop.

The pre-Workshop dialogues, which were hosted on the online platform, protocols.io, focused on generating advanced thought and engagement on antibody validation among the different stakeholders and producing preliminary recommendations on validation standards, information-sharing, drivers of adoption, and new technologies (including recombinant antibodies) for addressing the quality and consistency of research antibodies. With over 500 individual posts, the online dialogues formed the basis of pre-Workshop consensus building papers, which were provided to Workshop participants in advance of the conference[1]. Alongside these discussions, GBSI sent Workshop attendees a survey on the applicability and feasibility of each validation pillar or strategy for validation of antibodies used in specific experimental applications. The responses were summarized and included in the pre-Workshop consensus papers, which served as a resource for the Workshop and this Report.

The *Antibody Validation: Standards, Policies, and Practices* Workshop took place from September 25-27th in Pacific Grove, California at the historic Asilomar Conference Grounds. The overarching goals of the Workshop were:

- To support reproducibility in basic and pre-clinical research by improving the quality and use of research antibodies;
- To build consensus on validation standards for antibody-based applications and methodologies;
- To establish recommendations for the implementation and widespread adoption of validation standards.

The original themes of the workshop were:

- To create antibody validation standards in a fit-for-use, or application-specific manner;
- To define the responsibilities of producers and engage suppliers to use best practices for internal validation, transparency, and distribution of reagents;

1. The pre-Workshop Consensus-building Papers are accessible at https://www.gbsi.org/event/asilomar/. Accessed on November 8, 2016.

- To support acceptance and adoption of validation standards through funders, journals, certification, training, and proficiency;
- To integrate new technologies, including sequenced, recombinant antibodies, in support of reproducibility

The Workshop included sessions on the science behind antibody validation, recombinant antibodies, application-specific validation strategies, roles of producers and service providers, roles of journals, information-sharing databases, training and proficiency testing, and certification. In addition, consensus-building sessions were included to arrive at common approaches for validating antibodies and ensuring their broad adoption. The Workshop was highly participatory to encourage contributions by all attendees. The Workshop agenda is available at the event website, https://www.gbsi.org/event/asilomar/.

Two products were generated from this Workshop: 1) a consensus document that summarizes common principles for research antibody validation; and 2) the present Report, which presents the significant discussion points and recommendations from the Workshop. This Report is intended to present the perspectives conveyed during the Workshop and promote thoughtful consideration of the challenges of validation, validation practices, implementation approaches, and stakeholder roles and responsibilities for antibody validation. The Consensus Principles document and Report will inform GBSI's ongoing and future efforts towards defining antibody validation standards.

The third part of GBSI's strategy is the establishment of working groups to evaluate application-specific validation approaches, evaluate the roles and responsibilities of each stakeholder community, and implement strategies for wide-spread adoption of validation approaches and consensus principles. The outcomes of these Working Groups will be completed in Spring 2017 followed by a process to obtain wide community input, publish a paper describing community viewpoints, and work towards achieving broad implementation of the validation guidelines.

EXECUTIVE SUMMARY

Irreproducibility of scientific research is at the forefront of problems that limit scientific progress and the translation of basic and applied research (*Begley & Ellis, 2012; Freedman et al, 2015a; Freedman & Inglese, 2014; Hartshorne & Schachner, 2012*). In recent years, the scientific community has mobilized to address irreproducibility of research antibodies. Scientists, funders, and journal editors have initiated activities, such as establishment of resource databases and requirements for information-sharing, to promote transparency and reproducibility of research in which antibodies are used. However, a single set of community-accepted validation guidelines and standards that is sufficiently comprehensive and accessible to the entire biological research community has not emerged to date. GBSI and The Antibody Society sought to build on existing efforts towards agreement on a common set of standards and processes for validation of research antibodies in its 2016 *Antibody Validation: Strategies, Policies, and Practices* Workshop. This Report describes the key messages and recommendations that emerged during the Workshop.

KEY MESSAGES

- Developing validation standards that apply to all stakeholders is difficult because of differences in available resources among producers and users, varied and diverse application by the user community, and the availability of sufficient scientific knowledge about the antibody target. Although the development of standards for validating research antibodies generally is viewed as needed by the producer and user communities, what the standards consist of and how they should be developed differ by individual and stakeholder group.
- Stakeholders have a shared responsibility for promoting reproducibility in biomedical research by validating antibodies used in many basic and applied research efforts. Producers have additional responsibility in developing and maintaining high-quality and consistency during antibody production, communicating important reagent information and validation results, and promoting the transfer of this information with their product through distribution. Users have a responsibility to verify and independently validate antibodies before experimental use, train scientists on validation protocols and practices, and share information about validation results and antibody use.
- Information about antibody characteristics, production information, validation methodology, and validation results shared with customers improves researcher selection, verification, and further validation of research antibodies. Some of this information is already available to researchers through community resources (e.g., databases) and product information. Although attendees often differed in their views on the level of detail that should be shared, they ultimately identified a solution, which is to make validation methodologies and results accessible to end-users.
- Tracking and sharing both positive and negative results for antibodies would improve selection of high-quality, consistently produced antibodies, ultimately improving reproducibility of research.
- Suitability and performance of antibodies differ based on experimental use, specifically conditions and application. Antibodies that demonstrate high specificity in certain experiments may not in others. This observation holds true for antibody functionality in different conditions.
- Both fit-for-purpose validation (i.e., experiment-specific validation) and general validation strategies may be needed to assess research antibodies as commercial products and for specific use by researchers, respectively.

SUMMARY OF CONSENSUS PRINCIPLES

GBSI, in consultation with the Workshop Steering Committee, finalized and sent to Workshop attendees the Summary of Consensus Principles for Research Antibody Validation. (See Appendix) This document describes transparency, shared responsibility, and partnership as the fundamental principles that drive effective guidelines for antibody validation and reproducibility. GBSI highlighted the importance of building on the International Working Group on Antibody Validation framework for developing validation standards, the need for open and accessible sharing of information about antibody products and their validation results, the key roles and responsibilities of producers and users, and the broad interest in developing an antibody reporting system. The document concludes by linking these efforts, along with user training and producer certification, to the bigger issue of promoting reproducibility of biomedical research.

OVERVIEW OF NEXT STEPS

Following the conclusion of the Workshop, GBSI established Working Groups led by representatives from research antibody producer and user communities to develop the antibody validation standards, which will include a validation scoring system to detail antibody validation using specific applications. In addition, GBSI is working with producers to establish production standards and certification, and developing user training and proficiency testing tools.

SUMMARY OF RECOMMENDATIONS

During the Workshop, attendees discussed several recommendations for developing and implementing antibody validation standards. These recommendations will be considered by the Working Groups to determine whether and how they can be implemented.

- In consultation with producers and users from all relevant sectors, the GBSI Working Group should develop voluntary, performance-based antibody validation standards that promote application-specific testing, information-sharing and transparency. The standards should be derived from the pre-workshop, Workshop discussions, and published literature.

- Stakeholders should build on increasing scientific knowledge to improve standards, methodologies, and data analysis and interpretation for antibody validation. This information also could inform assessments on whether and to what degree certain validation strategies are predictive.
- A community-supported plan should be developed for addressing quality and performance questions about research antibodies, where sufficient scientific information is not available to assess specificity or any other characteristic in any experimental application or condition should be developed.
- A scoring system for antibody validation should be design according to the developed standards and enabling users to look at individual information (e.g., antibody characteristics and performance in specific applications, amount and quality of information shared, producer certification) and overall, cumulative quality.
- Producers should validate their products in the intended experimental applications and communicate these validation results, along with all validation and product information (including the dates of release of new lots and/or consistency in lot-to-lot performance), to users.
- Users should validate antibodies in the specific applications and experimental conditions of intended use, and communicate the results to producers, users, and other stakeholders.
- A producer certification system should be developed for antibody products based on clear quality control standards.
- Users should be trained on good validation practices and tested for proficiency.
- The stakeholder community should work together to determine how to leverage existing resources, such as databases and core facilities, to promote antibody validation standards and information-sharing.

WORKSHOP SUMMARY

BACKGROUND

Irreproducibility of scientific research is at the forefront of problems that limit scientific progress and the translation of basic and applied research (*Begley & Ellis, 2012; Freedman et al, 2015a; Freedman & Inglese, 2014; Hartshorne & Schachner, 2012*). Poor quality, inconsistent biological reagents contribute to the problem of irreproducibility of pre-clinical research. The $1.6 billion dollar research antibody market is expansive with more than 300 global vendors and over 2 million available reagents (*Baker, 2015b*). While many reagents are available for purchase, only a portion of commercial antibodies function as intended (*Berglund et al, 2008; Bradbury & Pluckthun, 2015a; Bradbury & Pluckthun, 2015b; Egelhofer et al, 2011; Michel et al, 2009*). Furthermore, some antibodies are suitable for use in certain applications and experimental conditions, which can result in end-users using inappropriate and ineffective antibodies for their specific research needs. Regardless of the challenge, incomplete or improper antibody validation has been identified as a major source of irreproducibility (*Baker, 2015b; Begley & Ellis, 2012; Bradbury & Pluckthun, 2015; Marx, 2013; Weller, 2016*). Improperly validated antibodies contribute to current challenges in reproducibility of life-science research and, in the case of clinical studies, can adversely affect participant inclusion in studies and clinical analysis of samples.

The scientific community recently has initiated efforts aimed at improving the quality and reproducibility of research antibodies. Several databases have been created to facilitate sharing of information about commercial antibodies and product reviews[2]. The National Institutes of Health (NIH) has developed one such repository of data on research antibodies and implemented requirements for promoting reproducibility of research[3]. Several journals have requested unique identifiers, batch information, and validation results for antibodies described in manuscripts. High impact journals, such as Science, Nature, and Nature Methods have provided forums for engagement and dialogue about antibody validation needs within the scientific community. (Uhlen et al, 2016)Finally, major antibody producers, such as ThermoFisher Scientific, have supported efforts to consider community-based solutions for increasing the quality and consistency of research antibodies (Uhlen et al, 2016). The Workshop sought to build on these efforts towards agreement on a common set of standards and processes for validation of research antibodies. This Report captures significant themes and recommendations that emerged from the discussions.

CONSIDERING ANTIBODY VALIDATION STANDARDS

The research antibody market has many different stakeholders (i.e., researchers, producers, service providers, funders, journal editors, beneficiaries of research) and underlying incentives (e.g., commercial, financial, technical). Each stakeholder makes decisions about antibody validation, use, and transparency and has a shared responsibility for improving reproducibility of research that involves antibodies (*Marx, 2013*). Using game theory, Workshop keynote speaker, Dr. David McAdams from the Duke University's Fuqua School of Business presented a framework for considering the benefits of using standards, harms if standards are not used or developed to address the problem adequately, and roles of each stakeholder in maximizing benefits and minimizing harms. He highlighted three primary purposes that standards play:

1) Aiding in decision-making and mistake avoidance.
2) Coordinating and leveraging the efforts of many.
3) Changing stakeholder behavior.

When applied to the challenges of developing standards for validating research antibodies, the primary, perhaps overriding, purpose of these standards is to influence stakeholder behavior such that they adopt policies and practices that promote use of validation standards and information sharing of reagent characteristics and validation results. Standards that positively meet the needs of all groups and minimize adverse effects on scientific practice and innovation are ideal. These types of standards are more likely to be adopted by users and achieve the benefits for which they were designed. However, negative incentives exist that can disrupt adoption of standards, suggesting enforcement and positive incentivization may be necessary to realize adoption. Thus, raising awareness, empowering stakeholders to make good decisions and demonstrate quality and expertise, and incentivizing behavior through enforcement are possible policy strategies that stakeholders can use to promote adoption of standards.

Key considerations for promoting adoption of standards includes:

1) The use of accumulative standards, those that iteratively evolve with current capabilities, ensures that standards use available technology and resources.
2) The use of conditional standards is appropriate given the underlying reagent and biological complexity, the application-specific context, and different roles and responsibilities of stakeholders.
3) Standards[4] that enable access to information on a resource or reagent, such as the use of databases, help to improve decision-making as long as they are open and interoperable.

2. Examples of antibody databases include: CiteAb (https://www.citeab.com/, Accessed on November 17, 2016), Antibody Validation Database (http://compbio.med.harvard.edu/antibodies/; Accessed on November 17, 2016), Labome (https://www.labome.com/method/Antibody-Validation.html; Accessed on November 17, 2016); Biocompare (http://www.biocompare.com/; Accessed on November 17, 2016); The Human Protein Atlas (http://www.proteinatlas.org/; Accessed on November 17, 2016); The Antibody Registry (http://antibodyregistry.org/; Accessed on November 17, 2016).

3. The National Institute of Health has an initiative in Rigor and Reproducibility which seeks to improve the design, performance, and reproducibility of biomedical research. The website is https://www.nih.gov/research-training/rigor-reproducibility. Accessed on November 17, 2016. The National Cancer Institute Antibody Portal is accessible at https://antibodies.cancer.gov/apps/site/default. Accessed on November 17, 2016.

4. In this sentence, "standards" does not refer to strategies that should be used to validate antibodies. Instead, it refers to a norm.

4) Certification allows users to select reagents that best fit their needs, saving research funds and leading to reproducible science, and encourages producers to manufacture of high quality products and transparency in the market.

5) Training and proficiency empowers researchers to demonstrate competency in antibody-based research.

OVERARCHING THEMES FROM THE WORKSHOP

Antibody validation is a complex problem, steeped in common and competing interests of its diverse stakeholders. Despite this complexity, several themes emerged from the Workshop:

- Developing validation standards that apply to all stakeholders is difficult because of differences in available resources among producers and users, varied and diverse application by the user community, and the availability of sufficient scientific knowledge about the antibody target. Although the development of standards for validating research antibodies generally is viewed as needed by the producer and user communities, what the standards consist of and how standards should be developed differ by individual and stakeholder group.
- Flexible standards are needed to enable their adaptation to different and/or new experimental uses, antibody production practices (e.g., recombinant antibodies), and available information shared.
- Overly complex, prescriptive, and/or restrictive standards that hamper product development and scientific progress likely will not be adopted.
- Newer methods for producing antibodies, such as recombinant antibodies, do not replace the need for validation. Regardless of how antibodies are produced, they should be validated by producers and users.
- Stakeholders have a shared responsibility for promoting reproducibility in biomedical research by validating antibody reagents used in basic and applied research efforts. Producers have additional responsibility in promoting the development of high-quality and consistency during antibody production and communicating important information about the antibodies they make and validation results. Some, but not all, attendees believed that producers are responsible for promoting the transfer of this information with their product through distribution. Users have a responsibility to verify and independently validate antibodies before experimental use, train researchers on validation protocols and practices, and share information about validation results.
- Information about antibody characteristics, production, validation methodology, and validation results shared with customers improves researcher selection, verification, and further validation of research antibodies. Some of this information is already available to researchers through community resources (e.g., databases) and product information. Although attendees often differed in their views on the level of detail that should be shared, they ultimately identified a solution, which is to make validation methodologies and results accessible to end-users.
- Tracking and sharing both positive and negative results for antibodies would improve selection of high-quality, consistently produced antibodies, ultimately improving reproducibility of research.
- Users communicate and receive information about antibody characteristics and validation strategies and results. Users can contribute to transparency efforts by including full details of their experimental and validation methodologies in manuscripts, providing information requested by journals, depositing their validation results and product reviews in existing databases and platforms, and providing feedback to companies about their antibody products.
- Armed with information about different antibodies, users can make informed decisions about which they purchase for their experiments by comparing products for their quality, production consistency functionality, and contraindications for different applications. In addition, users can identify specific batches that work better than others and trace their desired reagents from production to sale (or resale).
- Suitability and performance of antibodies differ based on use, specifically experimental conditions and application. Antibodies that demonstrate specificity in certain experiments may not in others. This observation holds true for antibody functionality in different conditions.
- Both fit-for-purpose validation (i.e., experiment-specific validation) and general validation strategies may be needed to assess research antibodies, as commercial products and for specific use by researchers.
- Use of precise definitions for antibody characteristics, validation strategies, and other key terms is critical to developing and adopting validation standards. Unclear definitions and differences in understanding of concepts led to several requests for clarification of terms, such as “routine quality control” versus “validation”, “antibody characterization” versus “validation”, or “specificity” and “selectivity”.
- The time, cost, and skill for any single validation strategy will determine the selection of validation strategies chosen by users and producers. Some validation strategies may not be feasible for stakeholders with limited funding, access to assays (e.g., proteomic arrays), core facilities, skill and expertise (e.g., mass spectrometry), scientific information, and time.
- Antibody validation practices and information-sharing are equally important for promoting reproducibility of research that relies on antibodies as experimental reagents.
- Market value, biological significance, and value to research are key considerations in determining whether and what type of new antibody reagents are introduced into the market.

PERCEIVED CHALLENGES FOR REPRODUCIBILITY OF RESEARCH ANTIBODIES

Participants described several problems that contribute to the problem of irreproducibility of research in which antibodies are used as reagents, including reselling of antibodies by different companies, lack of traceability of resold or reused antibodies in the market, inconsistent reporting of validation results, lack of validation standards with which to compare commercial antibodies, lack of uniform practices for validating antibodies, and variability in production quality across batches. Using a web-based polling tool, attendees were asked to rank which of these problems contributes most to the irreproducibility of research antibodies. (Figure 1)

The data described in the tables are not representative of all attendees; approximately half of the attendees participated in the live polling. The data does not capture verbally communicated perspectives or online comments. Discrepancies in the polling information and in-person or online comments are included in the Workshop Report to ensure objective summarization of the discussion.

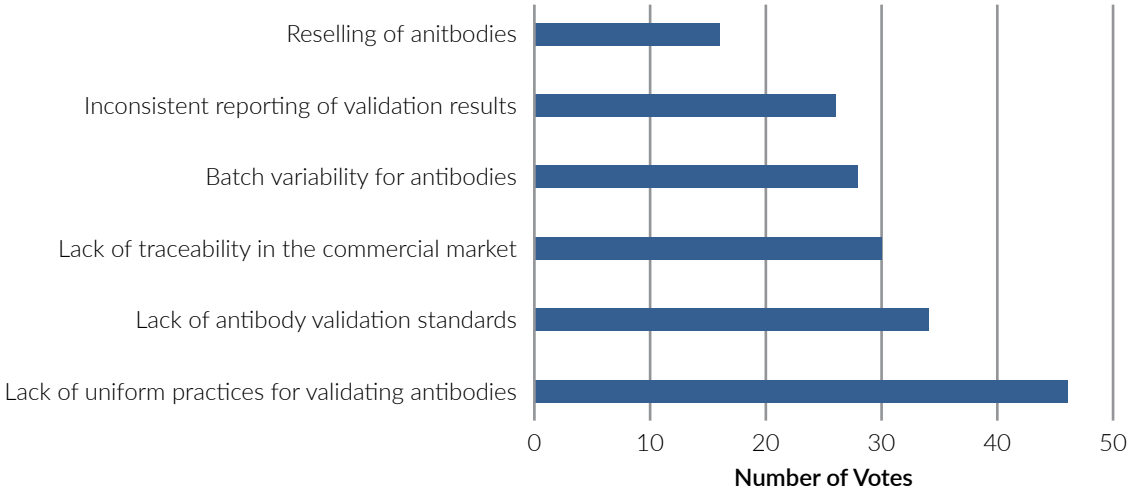

Figure 1. Identification of most problematic factors contributing to irreproducibility of research antibodies.

The most highly-ranked factor contributing to the irreproducibility of research involving antibody reagents problem is the lack of uniform practices for validating antibodies. Throughout the Workshop, questions were raised about the feasibility of developing uniform practices given available scientific data on antibody-antigen characteristics. The next most commonly selected problems with research antibodies were the lack of validation standards, traceability of commercial antibodies, batch variability, and inconsistent reporting of validation results. The least selected factor was reselling of antibodies. However, several attendees cautioned that the practice of re-selling may not be widely known or apparent to the research community. As the Workshop continued, the problem of reselling along with lack of product traceability was highlighted as a significant barrier to full transparency of antibody reagents that can inform consumer product choice.

PERCEIVED IMPORTANCE OF ANTIBODY CHARACTERISTICS TO BE VALIDATED

Antibodies have several properties that determine their utility as a reagent in biomedical research (functionality). These properties include its ability to bind a specific antigen (specificity), bind to other antigens that have similar epitopes (cross-reactivity), preferentially bind its target antigen in the presence of other antigens (selectivity), and recognize and bind small amounts of the target antigen (sensitivity). Also included are properties such as sequence identity (identity) and binding strength between the antibody and its antigen (avidity). Using the web-based polling platform, attendees were asked which of these antibody characteristics provide the most useful information about antibody quality and reliability. (Figure 2)

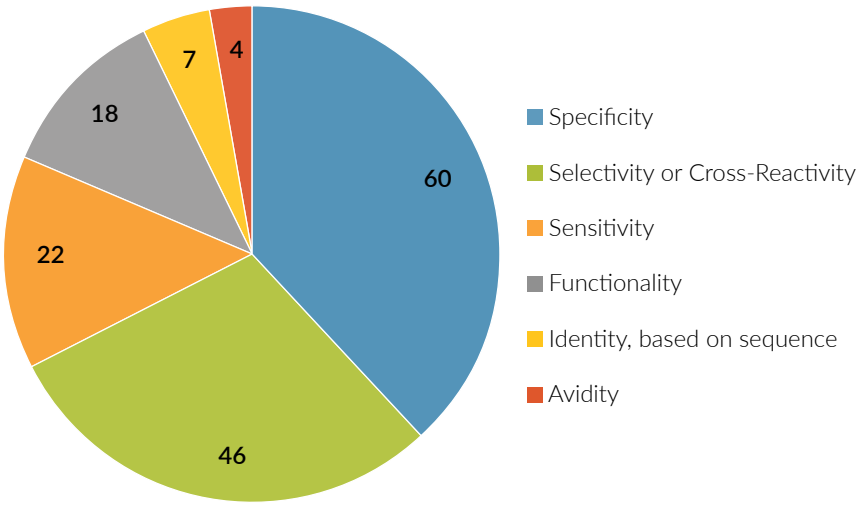

Figure 2. The number of votes for each antibody characteristic listed.

Antibody specificity and cross-reactivity or selectivity were selected as two of the most critical antibody characteristics to assess during validation. Many fewer attendees selected functionality and sensitivity as characteristics that need to be tested. However, attendees stated that "utility for specific assays," or fit-for-purpose assays is a more precise term for describing functionality.

VALIDATION STRATEGIES

Antibody validation refers to the process through which antibodies are deemed to function as intended and be specific, sensitive, and reproducible. Workshop attendees were split on their support for more general validation strategies and fit-for-purpose approaches. However, because function depends on many factors, including binding conditions, experimental application and context (e.g., in solution, cultured cells, tissues, or organism), and its own properties, validation may be done independent of experimental conditions (i.e., producer strategies) and within the specific experimental conditions (i.e., researcher strategies for testing antibodies in a fit-for-purpose or experiment-specific manner). Informing the selection of validation strategies and analysis of results involves generating scientific data that addresses existing knowledge gaps and can be reproduced by different researchers.

Validation strategies have different degrees of utility in assessing the performance and quality of antibodies used in different applications. This variability is caused by:

a) Imperfectly matched application-specific conditions with validation methodologies (e.g., application that require specific fixation chemicals whereas validation strategy does not require sample fixation);

b) The need to evaluate distinct antibody properties, such as binding strength for immunoprecipitation or tissue localization for immunohistochemistry; and

c) Effects within the sample or model system that affect antibody function (e.g., post-translational modification, cleavage of proteins and unexpected localization of proteins affecting antibody recognition, localization, and/or signal intensity).

Choice of validation strategies is essential for ensuring that the antibody is suitable for a given experimental application and that the most informative characteristics are examined.

Validation strategies that enable quantitative analysis of performance may reduce variability in results. Qualitative and semi-quantitative approaches, such as western blotting, are fraught with variability caused by methodological design, method of visualization, and time of exposure. While bands can be quantified using software, these results are not as reliable as quantitative assays, such as tissue microarrays (an orthogonal method) and flow cytometry, or semi-quantitative approaches, such as mass spectrometry.

PERCEIVED UTILITY OF ANTIBODY VALIDATION STRATEGIES

The International Working Group on Antibody Validation (IWGAV) qualitatively evaluated various types of validation "pillars" (also called strategies or approaches), suggesting specific experimental applications for which they would be informative and suitable for assessing antibodies (Uhlen et al, 2016). The pillars of validation include genetic approaches (specifically, knockdown and knockout), orthogonal or comparative methods, independent antibody-binding approaches (i.e., multiple epitope approaches), expression of tagged target protein, and immunocapture and mass spectrometry methods. Using the web-based polling tool, attendees were asked which validation strategies were most critical and least prohibitive from an expanded list that was developed from the IWGAV's pillars, pre-Workshop dialogues, and advice from the Steering Committee. (Figure 3)

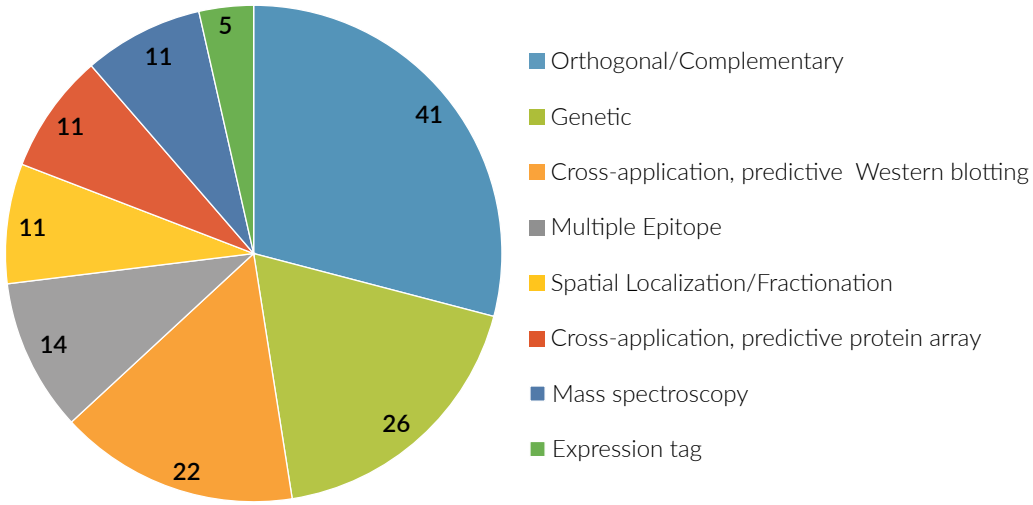

Figure 3. Figure 3. Number of votes for critical, non-prohibitive validation strategies.

More attendees selected orthogonal or comparative strategies as being both essential and not prohibitive, over any other approach. However, many attendees did not support the use of transcriptomics in predicting antibody performance, primarily because RNA expression does not always correlate tightly to protein abundance. The next most frequently selected strategies were genetic approaches and western blotting methods. Attendees caveated the use of genetic approaches as a preferred validation strategy because the specific methodology chosen may be expensive and/or technically challenging (e.g., knockdown of essential genes). Furthermore, genetic approaches are insufficient for validating antibodies to post-translationally modified proteins and modified

histones. The use of western blotting as a validation strategy received mixed responses among attendees, some favoring of its ability to predict specificity and cross-reactivity, while others highlighting its potential for inaccurate results, particularly for antibodies that recognize epitopes only present in a folded protein (e.g., where an antibody-binding site is only present in a properly folded protein).

The expression tag pillar received the least amount of support as a critical and feasible validation strategy. In addition, attendees cautioned against using: a) overexpression methods for validating antibody binding, specificity and cross-reactivity, which can result in false interpretations of antibody performance; b) peptide competition, which does not constitute an informative validation strategy; and c) omission of primary antibodies to evaluate the antibody specificity and antigen recognition, which also is not an informative validation strategy.

Strategies that are useful for testing antibodies used in specific experimental applications, such as spatial localization and immunocapture/mass spectrometry, were not highly selected by attendees. However, their utility in evaluating antibodies for certain applications was stressed during the open discussions. Furthermore, attendees who had experience working with mass spectrometry described it as one of the best and only technologies able to identify definitively the binding sequences, despite its inability to provide information about antibody cross-reactivity.

Attendees identified other validation methods, such as tissue microarrays and other sample arrays wherein the antigen's protein level varied. The major limitations of these array-based methods are their feasibility or utility for antibodies of uncharacterized protein targets and inaccessibility by a broader user-base. However, companies are investing in the development of these arrays as scientific tools. One type of array that received mixed responses from attendees is protein arrays. Attendees who favored protein arrays saw its value in high-throughput validation, while others highlighted its prohibitive factors, specifically the high cost and resources demands, and lack of sufficient knowledge about the peptides included in the array, raising questions about its ability to predict antibody binding for other experimental applications.

Table 1 summarizes the Workshop discussions on validation strategies and possible methods for each strategy.

Table 1: Antibody Validation Table		
Proposed* Validation Strategies		Current Methods for Each Strategy**
Binary | Use of methods to perturb (e.g., increase or decrease) the abundance of the antibody target	Genetic Strategies	RNAi-mediated knockdown CRISPR-Cas9 knockout or knockdown Traditional knockout models
	Stimuli-based Strategies (e.g., Activators, Inhibitors)	Activation or inhibition methods to increase or decrease the abundance of a post-translationally modified antigen
Expression Tag | Comparison of results against tagged and untagged target		Ectopic expression of tagged protein target for detecting the tag and target epitope
Titration-based Strategies | Evaluation of progressive increase or decrease of target specific-signal		Inducible expression systems Serial dilutions of purified proteins or cell lysates
Multi-sample Panels | Evaluation and comparison of signals in several samples.		Evaluation of antibody binding across multiple samples with a range of protein target expression (e.g., tissue microarrays)
Orthogonal/Comparative | Comparison of two or more methods to compare antibody results		Immunoprecipitation-mass spectrometry Mass spectrometry Protein and peptide arrays
		Biophysical and biomolecular-analysis (e.g., surface plasmon resonance)
Independent Antibody-Binding | Comparison of results of two antibodies against the same protein target		Binding of two independent antibodies to same protein target
Spatial Localization | Comparison of cellular and subcellular distribution of antibody signal based on available scientific knowledge of protein target		Microscopy (e.g., immunofluorescence) Flow cytometry
Cellular Fractionation | Evaluation antibody signal corresponding to subcellular compartments based on available scientific knowledge of protein target		Cellular fractionation and immunoprecipitation-mass spectrometry

*This table presents strategies discussed over the course of the Workshop, but is not indicative of their relative strengths, limitations, and broad suitability for antibody validation in different application and experimental contexts. These strategies and methods should be evaluated and updated periodically as information about cost-effective, feasible, and informative methods are identified (including new and existing validation methods) and as new scientific information about biological systems are produced.

**Asterisk indicates current representative examples of methods that could be used for each of the validation strategies.

GENERAL CONSIDERATIONS FOR ANTIBODY VALIDATION

- Reproducibility of validation results might be addressed by repeated examination using different laboratory reagents, several antibody lots, and operators or research teams. Although repeated validation assays could be expensive and time-consuming, such efforts may add confidence in the reproducibility of validation results and antibodies produced or used. For example, some producers have independent research teams validate antibodies to ensure reproducibility and reduce user variability.
- Optimizing the conditions of validation assays to reduce background enables researchers to evaluate more effectively the characteristics of importance (e.g., sensitivity and specificity). Reducing background noise ensures that any signal, whether caused by specific or non-specific binding, is attributed to the antibody being tested. Approaches for reducing background noise include antibody titration methodologies and methods for signal detection and visualization. For example, by changing the settings of signal detection or visualization equipment, faint, but real, signal may reveal potential non-specific or cross-reactive binding.
- Reagents and samples, such as curated, characterized, standardized, and validated knock out cell lines, could enhance validation efforts if provided to the scientific community, both producers and users of antibodies. These samples can serve as positive and negative controls, or well-characterized systems for assessing antibody characteristics. Government agencies and independent entities could play a role in funding, overseeing, or maintaining standard materials and samples for use in validation assays. However, few such resources exist, in part because of limited scientific knowledge. Investing in basic research to address key knowledge gaps could enable to identification and development of standardized resources for use in validation assays and improve efforts to create customized panels of standardized reagents for use in validation studies. Incompletely studied resources present significant challenges for ensuring replicability of validation results.
- Significant knowledge gaps exist about many human, mouse, non-human primate, and other model-system proteins, regulation of their expression, post-translational modification, and function in different contexts. Because antibody validation relies of extant knowledge of the protein target (e.g., its expression levels in different cell types, three-dimensional structure, post-translational modification, and existence as signal protein or part of a protein complex), developing validation strategies to test antibodies to uncharacterized proteins presents significant challenges. Similar difficulties may be observed with antibodies to post-translationally modified proteins.

APPLICATION-SPECIFIC VALIDATION STRATEGIES

Participants of the pre-Workshop dialogues and Workshop attendees supported the development of application-specific validation of research antibodies. During the Workshop, attendees were divided into seven groups, each corresponding to the experimental applications included in the IWGAV paper – Western blot, immunohistochemistry, immunocytochemistry, immunoprecipitation, flow cytometry, sandwich assays, and reverse phase protein array– to identify approaches for validating antibodies used in these applications. No group was formed for chromatin immunoprecipitation because it was sufficiently different from all other applications and no attendee had a high level of familiarity with the methodology.

Each group was asked to consider the following questions in their discussions about application-specific validation measures:

1) What antibody characteristics should be evaluated?
2) What suitable, practical, and feasible strategies can be used to evaluate a given characteristic?
3) What criteria should be used to analyze the validation results?

The outcomes of each group were described to all attendees, after which an attempt to identify consensus was initiated. Despite all efforts, the complexity of the problem and a shortage of time for small group discussions prevented a consensus about application-specific validation strategies from being developed. Also contributing to the problem were groups not completing the provided worksheets as requested and facilitators initiating consensus discussions about application-specific antibodies based on incomplete information. Despite these challenges, the group outcomes will serve as an informative start for the GBSI Working Groups that will recommend antibody validation standards and the scoring system.

Tables 2A-G summarize the outcomes of the small group discussions describing application-specific validation strategies for western blot (Table 2A), immunohistochemistry (Table 2B), immunocytochemistry (Table 2C), immunoprecipitation (Table 2D), flow cytometry (Table 2E), sandwich assays (Table 2F), and reverse phase protein array (Table 2G).

Table 2A.Western Blot			
Critical Antibody Characteristic to Validate	Validation Strategy	Purpose	Challenges
Specificity	Genetic Approaches	Correlate antibody signal and antigen abundance	The level of uncertainty of validation results varies and depends on the efficiency of the genetic method and existing scientific information about the target antigen. Proteins do not always migrate at the predicted molecular weight A single band may represent many proteins Need to evaluate relevance of multiple bands, if detected, to the underlying biology of the antigen (e.g., degradation products, cleavage, post-translational modifications, expression of closely-related targets, or natural variants caused by post-transcriptional or post-translational regulation)
	Orthogonal Approaches	Correlate antibody signal and antigen abundance in multiple samples	
	Independent Antibody-binding Approach	Correlate antibody signal using two distinct antibodies that bind to different (adjacent) sites on the antigen	
Sensitivity	Immunoprecipitation	Enrich for endogenous detection of low-abundance antigen	
	Subcellular fractionation	Enrich for endogenous detection of low-abundance antigen	

Table 2B. Immunohistochemistry			
Critical Antibody Characteristic to Validate	Validation Strategy	Purpose	Challenges
Specificity and Sensitivity	Tissue Microarray	Test for presence and abundance of antigen in experimental conditions using positive and negative controls	All reagents and samples must be biochemically validated to ensure they are appropriately characterized as positive and negative controls. The ability of and degree to which differences in signal across samples can be resolved. Gaps in scientific knowledge about protein expression and localization in different tissues. Fixation methods (e.g., paraffin embedded and frozen) affect antibody performance.
	Cell Index Arrays	Test for presence and abundance of antigen in experimental conditions using positive and negative controls	
Expected Functionality	Orthogonal Approach	Compare antibody using a test slide with unstained tissue from the producer and a picture of the stained image	

Table 2C. Immunocytochemistry			
Critical Antibody Characteristic to Validate	Validation Strategy	Purpose	Challenges
Specificity	Genetic Approaches	Examine the presence and absence of staining or signal in expected subcellular compartments	Must be conducted in a fit-for-purpose manner
	Spatial Localization Approaches	Examine signal or staining in subcellular compartments and correlate with a validated sample set and published information	
	RNA Interference	Examine the presence and absence of staining or signal in expected subcellular compartments.	
	Independent Antibody-Binding	Correlate antibody signal using two distinct antibodies that bind to different (adjacent) sites on the antigen	
Cross-reactivity	Overexpression	Examine the presence and level of signal[5].	
	Genetic Approaches	Examine the presence and absence of signal in a knockdown or knockout	
	Spatial Localization	Examine expected subcellular localization	
Sensitivity	Dilution Series Assays	Examine staining patterns across a range of antigen concentrations	

5. Overexpression systems are prone to aberrant results, such as mislocalization of the target protein, and misinterpretation of data.

Table 2D. Immunoprecipitation (and ChIP)			
Critical Antibody Characteristic to Validate	Validation Strategy	Purpose	Challenges
Functionality	Mass Spectometry	Examine enrichment and recovery of the antigen from the sample. (~80% recovery)	Validation has to be done using immunoprecipitation methods to test binding and enrichment of the antigen. A second strategy, which also may require validation, is needed to detect the enriched antigen(s) and determine antibody performance. Experimental conditions (e.g., denaturing conditions and sample type, such as serum versus lysate) affect antibody performance Need to assess binding affinity within a range that would enrich the antigen in an immunoprecipitation experiment Antibodies displaying different properties affect antigen recovery and enrichment, influencing validation results Closely-related proteins may be enriched for during validation. Controls must be of the same isotype as the antibody Monoclonal antibodies are more likely than polyclonal antibodies to experience interference caused by molecular interactions
	Western Blot	Examine enrichment and recovery of the antigen from the sample. (~80% recovery)	
Sensitivity	Dilution Series Assays	Correlate target abundance and enrichment	
	Expression Tag Approach	Correlate target abundance and enrichment	
Specificity	Genetic Approaches	Directly assess specificity of binding, correlate antigen abundance and known expression levels, and association of antigen binding partners	
	Dilution Series Assays	Directly assess specificity of binding, correlate antigen abundance and known expression levels, and association of antigen binding partners	
	Protein Arrays	Examine specific binding to intended antigen in the context of other potential proteins.	
	Spike and Recovery	Examine enrichment and recovery of antigen from solution	
	Expression Tag Approach	Correlate specificity of co-immunoprecipitation of antibody with anti-tag antibody	
Affinity	Surface Plasmon Resonance, Octet, Kinexa	Measure binding strength of the antibody for the antigen and measure binding off-rate to determine whether the antibody will work in an immunoprecipitation assay	

Table 2E. Flow Cytometry			
Critical Antibody Characteristic to Validate	Validation Strategy	Purpose	Challenges
Reactivity (Functionality)	Flow Cytometry	Examine use with reference material, and positive and negative controls.	Assumptions that antibodies against cell surface proteins are already validated through collaborative epitope mapping and the Human Leukocyte Differentiation Antigens (HLDA) Workshops[6] Antibodies to intracellular antigens, which are accessible only after permeabilization, have not been validated extensively using flow cytometry. Labels on primary and secondary antibodies can affect stability, efficiency, strength, and activity.
Specificity	Flow Cytometry	Correlate expected expression pattern on cells with and without the antigen and signal	
	Flow Imaging or Immununofluorescence microscopy	Correlate spatial localization of the signal with scientific information	
Selectivity	Flow Cytometry	Correlate signal on predicted cell subtypes based on scientific knowledge using gating strategies	
	Antibody Dilution and Spike Recovery of Antigen	Demonstrate linearity of antibody-antigen binding	

6. The Human Leukocyte Differentiation Antigens (HLDA) Workshops were a collaborative community effort that leveraged specialized expertise to validate antibodies against surface molecules on leukocytes that are important for diagnostic and research purposes.

Table 2F. Sandwich Assays			
Critical Antibody Characteristic to Validate	Validation Strategy	Purpose	Challenges
Cross-reactivity	Unassigned Approach	Examine if antibody binds only the antigen and no other antigens	Assay relies on two antibodies to act together to capture and detect the antigen in a fixed matrix. Need to demonstrate affinity within a range that would enable capture and enrichment. The purity and stability of the antibody affects antibody performance. Information about the antigen and binding epitopes are important when selecting and evaluating antibodies for capture and detection. Labels of antibodies can affect stability, efficiency, strength, and activity. Epitopic diversity of polyclonal antibodies used for capture and detection affect suitability.
Specificity	Immunoprecipitation/ Mass Spectometry	Examine if the antibody binds the intended antigen in experimental conditions and with necessary kinetics	
	Immunoprecipitation/ Western Blot	Examine the antibody binds the intended antigen in experimental conditions and with necessary kinetics	
	Genetic Approaches	Correlate antigen abundance with known expression levels	
	Inducible or overexpression	Correlate binding as antigen abundance changes	
Affinity	Dilution Series Assays	Titrate the antibody to examine the range at which the antibody can function	
	Surface Plasmon Resonance	Measure binding strength of the antibody for the antigen and measure binding off-rate to determine whether the antibody will work in a sandwich assay	
Selectivity	Antibody Dilution and Spike Recovery of Antigen	Demonstrate linearity of antibody-antigen binding	

Table 2G. Rapid Phase Protein Array			
Critical Antibody Characteristic to Validate	Validation Strategy	Purpose	Challenges
Specificity	Label-free Spectrometry	Correlate antigen abundance using tissue or sample profiles	Limited use application, which may result in limited validation by producers.
Sensitivity	Genetic Approach	Correlate variations of antigen abundance with signal intensity using overexpression or inducible expression	
	Spike Recovery	Correlate variation of antigen abundance with signal intensity using spike and recovery of antigen	
Selectivity	Tissue Panel	Correlate antibody signals in panels of tissues with varying amounts of antigen	

GENERAL CONSIDERATIONS FOR APPLICATION-SPECIFIC VALIDATION

- All results of validation tests are important to track and communicate. Results that demonstrate the antibody does not function as expected, bind its antigen with high specificity and selectivity, or recognize its antigen are equally as informative as results that demonstrate specificity, selectivity, functionality, and high affinity. **Tracking and sharing both positive and negative results for antibodies would improve selection of high-quality, consistently produced antibodies, ultimately improving reproducibility of research.** Furthermore, validating antibodies in several applications and conditions improves producer and user understanding about the cause of negative results (i.e., whether specific conditions adversely affect validation results rather than antibodies that are truly not suitable for use in certain applications.)
- Polyclonal, monoclonal, and recombinant antibodies must be validated for suitability in different applications. Unlike polyclonal and monoclonal antibodies, recombinant antibodies are molecularly-defined entities that can be customized to specific applications and experimental conditions. These features can improve selection of antibody-specific validation methodologies and analysis of validation results for specificity and selectivity. Furthermore, attendees suggested that panels generated from selected recombinant antibody clones could be used in independent antibody validation methodologies.
- Development of application-specific validation standards involves active engagement and dialogue with producers, users, and experts on each experimental application. The Working Groups that GBSI is establishing as part of the third phase of their antibody validation efforts can provide leadership in stakeholder engagement and dialogue.
- At the beginning of the Workshop, attendees discussed the need for a scoring system for application-specific information. Such a system could include all relevant information about validation tests, updates, and findings as new results are shared.

INFORMATION-SHARING

Transparency and information-sharing were common themes throughout the Workshop. Attendees discussed information-sharing in general terms and with respect to specific stakeholder groups. Stakeholder-specific efforts and needs for transparency of antibody information, including validation methodologies and results, were considered within the context of promoting adoption of validation standards.

GENERAL SUGGESTIONS

Attendees provided several general suggestions for promoting transparency by relevant stakeholders, primarily by producer and user communities.

- Producers and users have shared responsibility in validating antibodies and communicating results. Producers that validate antibodies in defined experimental applications provide customers with information about applications in which the antibodies can be used and those in which antibodies are contraindicated. Researchers provide information about antibody performance based on their validation of antibodies in experimental applications and conditions of intended use.
- Attendees generally agreed that the following information should be shared with all stakeholders:
    - *Antibody Information* | Supplier name, catalogue number, clone number, batch or lot number, antibody name, species of origin (e.g., mouse, rabbit, and goat), preparation method (i.e., monoclonal, and polyclonal, recombinant), antigen information, Research Resource Identification (RRIDs).
    - *Validation Information* | Antibody characteristic tested, validation strategy, validation methodologies, assay conditions (e.g., native or denaturing), positive and negative controls, critical sample information (e.g., cell or tissue type, organism, lysate, purified protein, growth or stimulation conditions).

  However, attendees did not agree about whether raw data or a statement of findings of validation tests should be shared.
- All stakeholders can play a role in raising awareness of the problem associated with research antibodies, highlighting the long-term effects on reproducibility of science, promoting adoption of validation standards, and encouraging greater information-sharing about research antibody characteristics and experimental uses.
- Journal editors and funders should use community-derived standards for antibody validation as a guide during manuscript or proposal review.

JOURNAL ROLES

Scientific journals play a crucial role in promoting information-sharing and driving adoption of standards that ultimately increase transparency and improve reproducibility. Nearly two dozen high impact scientific publishers request, if not require, the authors to provide some level of information about antibodies included in manuscripts. This information ranges from a simple statement of findings about antibody validation to inclusion of a unique identifier for the reagent.

During the Workshop, editors of high-impact scientific publishers discussed the need for increased transparency by including all experimental details and reagent information in the methods sections, including those associated with validation of antibody reagents, instead of simply referencing previously published methods sections. Standardizing methods sections, requiring links to publicly-available resources, distributing checklists of information needs are three suggested approaches for ensuring full experimental and reagent detail is included in manuscripts.

In addition, journal editors can require submission and/or easy access to raw experimental and validation data. Currently, journals such as Science, require submission of raw data to allow other researchers to analyze the data all for reproducibility in science. Details about the kinds of data, file types, database standards, and data sharing platforms were not discussed, but were raised as important for promoting data sharing and access.

Journal editors cautioned Workshop attendees to consider the mission and role of publishers in antibody validation and reproducibility:

- Because journals provide a service to the community and do not have a regulatory capacity, they rely on community leaders to define standards for information-sharing and validation.
- Although some scientific journals have professional editors, many publishers rely on part-time editors, most of whom have full-time employment in industry, academia, or at other research institutions. Furthermore, editors and peer reviewers are not necessarily familiar with appropriate validation strategies for testing antibodies generally and for specific applications. Supplementing the available expertise with additional experts, such as with a topic-specific experts panel, may be cost prohibitive for many publishers, particularly if such panels are needed for all reagents and methods that would benefit from additional expert review. A less expensive approach is to have journal editors leverage existing relationships with scientists who are familiar with antibody validation and existing curated and easy-to-access databases and data depositories of information about antibody quality, product consistency, and validation results.
- Research is communicated well-before the publication stage, mainly in institutional review bodies, grant proposals, conference presentations, and symposia talks. Journal editors can enable communication of antibody information and validation results, but their ability to enforce improperly validated antibodies is limited because the work has been completed already and authors may choose to publish in journals that do not require stringent reporting of antibody reagents.

FUNDER ROLES

Government and non-governmental funders play a variety of roles in promoting antibody validation standards and sharing of validation results. Dr. James Anderson from the National Institutes of Health discussed the role of funders, specifically focusing on the NIH and its Rigor and Reproducibility Initiative.

Many funders are not regulatory bodies and may not be able to set or enforce validation standards. However, they do have several levers at their disposal to promote information-sharing and validation of research antibodies. Funders can leverage their abilities to request more information or reject proposals that do not meet requested requirements, include contractually-binding requirements in their grant or contract awards, and remove funding from awardees who do not comply with the requirements. Some of these requirements could include following and communicating validation standards, and training on antibody validation.

Funders can create community platforms through which data, reagent information, or specific reagents can be shared within researchers (e.g., NIH 3D Print Exchange). However, the costs for maintaining these repositories and/or curating and validating information or reagents in repositories may be prohibitive. Development of portals wherein data about reagents such as antibodies can be deposited and shared may be less costly, but still requires curation of the deposited information. Organizations have supported field-specific repositories and reagent validation efforts. Broadening these efforts may enable broader access to antibody information and validation results.

The Rigor and Reproducibility Initiative of the NIH seeks to improve biomedical research by requiring information about the authentication of select biological reagents, such as methodologies for validating reagents, in grant applications. Proposal reviewers are asked to evaluate these methods and the applicants' expertise during the review process to ensure the investigator has a process in place to properly validate the experimental reagents before initiating experiments. Although these reviews do not affect the overall score of the proposal, reviewers can assess whether the applicants have the requisite expertise and request corrections without punishment or disqualification.

In general, few funds are allocated to the development and maintenance of collections, databases, and repositories. Increases in funding for curation and maintenance of these resources would enable greater functionality, access, and accuracy in the information and services provided.

Finally, organizations, that do not fund biomedical research, have supported social science and community-based efforts on reproducibility and/or validity of life science research. An analogous example is the Laura and John Arnold Foundation's initiative on research integrity, which seeks to improve reproducibility.

PRODUCER ROLES

The producer stakeholder group is very diverse, encompassing developers and manufacturers (together referred to as "producers"), Original Equipment Manufacturer (OEM) companies, and resellers. Each of these stakeholder groups are associated with different challenges to information-sharing and transparency.

The information provided by producers varies widely, ranging from information about only the product to information about the product (e.g., target antigen, antibody sequence, batch and lot information), recommended applications, contraindicated applications, validation results, and customer reviews. Most companies provide product information to consumers about the antibody and its expected functionality. Some producers also share information about strategies they use to validate their antibody products, validation results, and applications for which the antibody has been found suitable. Fewer companies provide easily accessible information on the different types of strategies they use to validate antibodies[7]. Some companies welcome consumer feedback of their products to update their antibody selections and/or enable customers to make informed decisions during antibody purchase. Workshop attendees stated that customer reviews do not replace validation, but they would enhance producer product offerings and user selections. When products fail quality control or validation, reputable producers demonstrate responsible practices by removing the antibody from the market, leave product information in the catalogue to ensure users can access cited information, and/or communicate the failure and cause to users.

The variability of information provided by producers and the strong inclination of companies to cite protection of proprietary information as a reason for limited information-sharing adds to the existing challenges of antibody validation. Although producers generally agreed that information about the antigen and antibody specificity should be shared, they disagreed on whether to release sequence of recombinant antibodies because of a lack of protection of intellectual property. However, some users advocated for sharing sequence information of recombinant antibodies, referencing benefits to improving traceability of antibodies in the market, preventing loss of critical reagents from the market, and enabling the development of new scientific tools, such as chimeras and targeted probes for functional studies. The legal landscape for protecting sequence information is complex and depends on the identification and development of the reagent, including its derivation from nature. Attendees discussed intellectual property protection and costs at length during the meeting. They suggested sequence accession numbers or protected sequence repositories as possible alternatives to open sharing of full antibody sequences. However, most attendees agreed that availability of sequence information does not eliminate the need for validation.

Resellers and OEM companies increase accessibility of antibodies to users, but they often do not provide the same level of information about the antibody product. Recent studies indicate that relatively few antibodies included in publications could be uniquely identified (*Vasilevsky et al. 2013*) and show identical results from seemingly-different antibodies purchased from different companies[8], suggesting

7. Abcam, A Guide to Antibody Validation. Accessible at: http://www.abcam.com/primary-antibodies/a-guide-to-antibody-validation. Accessed on November 13, 2016.

8. Dr. Andrew Bradbury shared primary validation data on protocols.io of an antibody purchased from different companies.

that antibodies sold through OEM companies and resellers lack sufficient information tying them back to the original product and its associated information, including batch number. This insufficiency of data associated with widely distributed antibodies, many of which are renamed or rebranded from the original product, highlights challenges of product traceability and transparency. Producers had mixed views about the degree to which traceability contributes to irreproducibility, while users agreed that lack of traceability and unique identifiers hindered selection of antibodies and some validation strategies (e.g., independent antibody-binding approaches).

VALIDATION SCORING SYSTEM

Workshop attendees generally agreed that a system for scoring of antibodies for their overall quality and performance, and individual characteristics would be useful. The scores would be based on the level of information available about the antibodies and results of application-specific validation. The system's ability to adapt to new scientific knowledge and technologies would improve its long-term utility and sustainability. Attendees suggested that this type of scoring system would enable users to select antibodies that matches their specific needs. However, it does not alleviate the need for users to validate the antibodies in their intended experimental application(s) and conditions. Producers agreed that a scoring system also could convey information about use of best production practices. Despite the high degree of support for a scoring system, Workshop attendees were unclear about the level of detail that would be needed to inform the scoring criteria. Scores that were generated using highly specific validation assays may not be informative for antibody suitability in other applications and/or conditions.

A new scoring system could be added to an existing database of antibody information or established as a new system of communicating information about antibodies. Workshop attendees highlighted the need for a single curated database wherein validation results, and methodologies used, could be shared to users. In the Consensus Principles document, the proposed scoring system was titled 'GBSI Score'.

EXISTING ANTIBODY DATABASES

During the past several years, several databases were created to improve transparency of information about research antibodies and enable users to make informed decisions about these reagents. Each database includes different information about antibodies, but several: a) link antibody information collected from a variety of sources; b) link antibodies to applications, species, and experimental conditions; c) describe scientific information that support antibody validation; d) list antibody validation strategies and standards; e) rely on stakeholders to access the databases and deposit information into the databases; or f) gather data on antibody use and performance in different experimental applications and conditions. Some databases also collect user reviews, which provide qualitative and subjective feedback on antibody quality, functionality, suitability to applications, and performance.

Analysis of the data collected in these databases could facilitate the development of standards for information-sharing. Furthermore, these data analyses could help to identify potential redundancies in product specifications and uncover valuable information driving more producers to validate their antibodies and/or release unique product identifiers.

Figure 4 describes existing databases for improving reproducibility of research antibodies.

The Antibody Registry
• Use of RRIDs (traceable product identifiers) and two-way verification linking antibodies, product sheets, and scientific articles (as data or information sources, and/or citations). • Link between application, specific products, and producters. • Limitations: Reliance on a) broad community use; and b) RRIDs, which cannot distinguish product redundancy caused by reselling, insufficient information about lot numbers, and inaccurate catalogue numbers. • Benefits: Platform for journals to link to and extract data about antibodies.
CiteAB
• Antibody search engine that uses citations to agregate data from publications, describe antibody use, and indirectly show antibody performance. • Tool that enables users to select antibodies that best match their experimental needs and producers to understand their customer base, including experimental uses. • Limitations: a) unclear whether citation rate effectively indictes antibody performance or simply shows reagents most often cited in publications; b) reliance on accuracy and accessibility of catalogue numbers; and c) antibody validation standards are not directly assessed. • Benefits: a) ability for suppliers to add citations to thier product entries; and b) ability for developers of antibody panels to link database information.
Human Protein Atlas
• Database that includes information about human proteins and transcripts, including primary expression data, results of polyclonal antibodies tested in several tissues, and antibody validation data. • Antibody validation score includes suitablity of antibodies for different applications enabling informed decision-making. • Limitations: a) antibody validation score is qualitatively and subjectively determined; b) validation methodologies may not optimize for low background signal; and c) specific isoforms are not extensively validated. • Benefits: future plan to validate antibodies using IWGAV pillars.
BioCompare
• Directory that aggregates product information from suppliers, editorial reviews of product, antibody functionality in applications and conditions, enabling cutomers to search for antibodies based on product specifications and reviews. • Limitations: a) difficult to scale up; b) reliance on community use; and c) unable to describe product redundancies.
Labomb
• Database of mannually-curated monoclonal antibodies, organized by gene identification numbers and linked to antibodeis, antigens or epitopes, and antibody use in literature and product sheets. • Platform to select select antibodies based on use in literature and product information • Limitation: unable to describe and resolve product redundancies.
Resource Identification Portal
• Platform for finding or generating unique identifiers for antibodies, organisms, cell lines, and tools. • Limitation: Relies on users to submit the unique identifier and include protocol details to allow for reproduction.
Antibody Validation Database
• Database of application-specific validation of research antibodies • The database works with expert labs to validate antibodies.

Figure 4. Brief descriptions of existing databases for sharing information about research antibodies.

ANALOGOUS EXAMPLE: CONSUMER REPORTS

An example analogous to the antibody scoring system concept is Consumer Reports, which rates overall product quality, reliability, functionality, and performance based on established criteria and includes expert reviews. Ratings can be viewed individually for each product or comparatively across several related products. Consumer Reports provides independent review of commercial products and services that can enhance user decision-making about the product and demonstrate (or refute) company claims of performance and quality. The example described during the Workshop was of home improvement tools, which are given a cumulative review and rating that is based on product specifications, customer ratings, and scores for individual product characteristics and/or capabilities based on its availability and performance. Attendees explored whether this format could serve as a possible format for developing a scoring system for antibodies.

KEY CONSIDERATIONS FOR A SCORING SYSTEM

Within the context of research antibodies, products could be scored based on a defined set of criteria for overall quality and performance, for individual characteristics, and for functionality in experimental applications. Key considerations for developing and implementing an antibody scoring system include:

- Because antibody characteristics, such as specificity and cross-reactivity, are subject to the specific conditions of experimental applications, scorings of individual characteristics must be evaluated in an application-specific manner. Furthermore, all details for this evaluation, including validation strategies, methodologies, and criteria must be communicated with the score.

- The scoring system could provide an overall, cumulative rating and individual scores for specific antibody characteristics, performance during validation, level of transparency, producer certification, and other information as relevant.

Provisions must be made to ensure that no one can "game" the scoring system with misleading inaccurate assessments.

PRODUCER CERTIFICATION

Workshop attendees discussed the need for and complications associated with producer certification. Certification of industry tends to focus more on critical processes such as research and development or manufacturing. Although industry certification ensures minimal process standards are met, the possibility exists that antibody products with variable quality and consistency can be produced even in certified development and manufacturing facilities. Workshop attendees generally agreed on the need for quality assurance standards for products, validation standards, and quality standards for producers all of which could be certified through different mechanisms. Considering this, attendees suggested options for producer certification.

Options for Producer Certification

- Establishment of a **regulatory system** or **ISO (International Standards Organization) standards based** on minimal standards of biological material production quality control. These options could enable reporting of poor quality products or user complaints to identify potential production problems, their causes, and need for and type of corrective actions.
- Development of **competency and quality standards** for antibody products. Certification would be on the product and brand of product.

TRAINING AND PROFICIENCY TESTING

Workshop attendees agreed that antibody validation for specific experimental applications and conditions is both valuable and needed. Many attendees were not confident that junior scientists, who have less knowledge and experience than senior scientists, would be able to select and validate antibodies effectively without training and proficiency testing. Furthermore, attendees agreed that regardless of years-of-experience, all users should be trained and tested for proficiency despite competing priorities, such as securing funding and publishing scientific papers. However, funding requirements and job marketability are two potential incentives for promoting training and proficiency testing among all users. For example, consequences for not training organizations could be loss of funding, pause or suspension of funding, requirement to retest or retrain.

The approaches and content of training varied among attendees, with some supporting more passive strategies such as online training and technical guides, and others supporting more active, hands-on strategies. Although classroom-based training on experimental design, importance of positive and negative controls, and equipment use was discussed, simply learning information does not translate to building laboratory-based skills. For this reason, attendees agreed that the most effective approach for training on antibody validation is hands-on, a concept that is supported by pedagogy experts. (*Smith, 2005; Michael, 2006*) In addition to format, content of training programs was discussed. Among the topics included are protocol design for validation, antibody selection, use in experimental applications, data analysis and interpretation, and best validation practices. Several examples of training programs and proficiency testing exist within the biomedical and clinical research disciplines, including required training by funders and regulators.

Options for Training Programs

- Potential formats for training vary from passive to active learning. Optional formats include:
  - Training through apprenticeships allows for hands-on training for antibody validation. However, the content of this training would vary unless a standardized curriculum is developed.
  - Remote or online courses that raise awareness and transfer knowledge about antibody validation.
  - Classroom-based training for antibody validation.
  - Course with a laboratory component that ties together classroom and experiential training.
  - Just-in-time training to educate scientists about antibody validation quickly.
  - Satellite workshops either with or without a laboratory component that ties together knowledge transfer and experiential training.
- Regardless of format, several options for who should develop the training were discussed: an independent third party, academic experts, academic experts in consultation with providers, a regulatory organization, a certification organization, and antibody affinity group.
- Trainers could be an interested stakeholder, trained trainers, professional trainers and inspectors, academic experts, or independent third party expert. Regardless of who educates, trainers with demonstrated training experience, proficiency in antibody validation, and experience with conducting experiments that rely on antibodies have the requisite familiarity with the content serve as trainers.

Options for Proficiency Testing

- Pre- and post-tests to demonstrate knowledge gain.
- Experimental testing using blinded samples. Although this approach has been used by many sectors to demonstrate proficiency, it was not viewed favorably by many attendees.

RECOMMENDATIONS FOR ANTIBODY VALIDATION

During the Workshop, attendees discussed several recommendations for developing and implementing antibody validation standards. These recommendations will be considered by the GBSI working groups to determine whether and how they can be implemented.

Recommendations on Antibody Validation

- In consultation with producers and users from all relevant sectors, the GBSI Working Groups should develop antibody validation standards and a related scoring system. The standards should be derived from the pre-Workshop and Workshop discussions.
- Validation standards should be voluntary and performance-based, rather than mandatory and prescriptive, to enable their long-term adoption and adaptability to advances in science and technology.
- A community-supported plan, process or approach should be developed for addressing quality and performance questions about discovery antibodies, where sufficient scientific information is not available to assess specificity, selectivity, or any other characteristic in any experimental application or condition.
- A scoring system for antibody validation, based on the standards developed, should be created that enables users to look at individual information (e.g., antibody characteristics and performance in specific applications, amount and quality of information shared, certification of producers) and overall, cumulative quality.
- Polyclonal, monoclonal, and recombinant antibodies should be validated, regardless of preparation method.
- Stakeholders should build on increasing scientific knowledge to improve standards, methodologies, and data analysis and interpretation for antibody validation. This information also could inform assessments on whether and to what degree certain validation strategies are predictive.

Recommendations for Stakeholder Roles and Responsibilities

- Producers should validate their antibody products in experimental applications and communicate these validation results, along with all validation and product information (including the dates of release of new lots and/or consistency in lot-to-lot performance), to users.
- Producers should receive certification for their antibody products based on clear quality control standards.
- Users should validate antibodies in the applications and conditions of intended use, and communicate the results to producers and users.
- Users should be trained on good validation practices and tested for proficiency.
- The stakeholder community should work together to determine how to leverage existing resources, such as databases and core facilities, to promote antibody validation standards and information-sharing.

CONCLUSIONS AND OVERVIEW OF NEXT STEPS

Antibody validation presents a significant challenge to promoting reproducibility of biomedical research. Unlike other fields, where development of standards might be simpler, the inherent variability in antibody function based on basic biology and experimental context is significant. The Antibody Validation: Strategies, Policies, and Practices Workshop provided an important forum wherein representatives from all stakeholder groups could participate, voice their opinions, and discuss recommendations for how to develop antibody validation standards and drive their broad adoption. Furthermore, the Workshop enabled stakeholders to identify critical information that would enable users to make informed decisions about their antibody selections and producers to refine product information based on user validation results. The immediate outcome of the Workshop was the Summary of Consensus Principles for Research Antibody Validation that was shared with Workshop participants in October 2016. The longer-term outcomes, which will draw on the information provided in this Report, is the establishment of antibody validation standards, a framework for an antibody scoring system, online user training and proficiency testing tools, and certification process for producers. To accomplish the longer-term outcomes, GBSI has established Working Groups of representatives from research antibody producer and user communities to deliberate and develop the antibody validation standards and scoring framework. During this 6-month period, the Working Groups will engage with academic scientists, publishers, and funders to solicit their input and leverage their expertise.

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APPENDIX A:

CONSENSUS PRINCIPLES FOR RESEARCH ANTIBODY VALIDATION: ASILOMAR ANTIBODY VALIDATION WORKSHOP

Research antibodies are among the most widely-used reagents in the life sciences. Improperly validated antibodies contribute to current challenges in the reproducibility of life-science research and, in the case of clinical studies, can adversely affect participant inclusion in studies and clinical analysis of samples.

In September 2016 at the historic Asilomar Conference Grounds in Pacific Grove, California, users and producers came together at the Antibody Validation: Standards, Policies, and Practices Workshop. Participants moved towards consensus around key approaches to research antibody validation—the process to create validation standards, including a scoring/rating system for antibodies, producer certification to the standards, and user proficiency for antibody validation. Moreover, the Global Biological Standards Institute (GBSI) affirmed its commitment to lead the process going forward to expand the consensus needed to establish validation standards and the related producer and user systems required to ensure implementation of those standards. To that end, GBSI will create focused working groups comprised of users and producers to complete the work initiated at Asilomar on a pplication-specific validation guidelines.

The fundamental elements that will drive effective guidelines are **transparency, shared responsibility**, and **partnership**. With these elements in mind, GBSI will encourage and support the user and producer communities to implement antibody validation policies and procedures based on the following principles agreed on at the Asilomar Workshop:

1) Detailed antibody validation strategies and standards that build upon the conceptual framework put forward by the International Working Group on Antibody Validation will be developed through the formation of focused, application-specific working groups. While not necessarily exhaustive, these strategies and methods will provide sufficient detail to enable the validation of specific molecular characteristics of antibodies. The degree to which these strategies increase confidence in the quality and suitability of the antibody will depend on the experimental conditions under which validation is conducted and the amount of scientific information about the protein target and validation method, and should be updated as new scientific knowledge is gained and biotechnologies are developed and validated.
2) Validation of all lots of antibodies manufactured using strategies that correlate best with the intended product applications would significantly inform users about the appropriateness of the antibody being purchased. In their product information materials, producers should consider describing the experimental applications and contraindicated applications for the antibody product based on the company's validation and user comments.
3) All information that can be provided by the producer about the validation approach, laboratory protocol, reagents, standardized samples (if used), and, if possible, data (both raw data and/or analyzed results) to users with their product information and/or material transfer agreements would also greatly inform users. The laboratory protocol provided would not need to include any proprietary reagents, but could include reagent lot/batch numbers, to ensure that users can replicate the producer's validation data and gain confidence that the product works as expected from the catalog information.
4) Access to antibody and validation information that follows the product through re-sale (if applicable) is an important source of data. Information about the clone number, molecular properties, species, type (polyclonal, monoclonal, or recombinant), antigen, and any other distinguishing or uniquely-identifying information of the antibody product should also be made available.
5) Users have an important responsibility to examine all available information about the antibody product before purchase, including any user-/community-antibody databases to determine: a) whether the antibody is intended for their specific application and not contraindicated in their application; and b) whether product consistency and quality is maintained during production through user-reviews, and has not been removed from the market because of poor, inconsistent quality.
6) Even with access to the producer's validation data, it is still important for the user to independently validate the antibody using the application and conditions for its intended use within their lab setting. Included in this "fit-for-use" validation approach is sample type (e.g., cell lysates, tissues, and purified protein), native or denaturing conditions, antibody and antigen concentration, and other relevant experimental conditions.
7) Communication by users of all relevant information about their experimental methods (including detailed, not referenced methods) for validating antibodies and antibody information (including molecular properties, clone and lot numbers, the producer or reseller, any distinguishing or unique-identifiers, and antigen information) in published articles and other methods of communication (e.g., conference posters) could advance the future reproducibility of studies by subsequent researchers.
8) There was consensus for users and producers to work together to develop and implement a ratings/scoring system that enables users to identify and compare antibodies. To be informative, the validation strategies, protocols, and evaluation criteria used to test antibody characteristics must be clearly defined and presented as part of the scoring process. Consistency in the validation strategies used and transparency of information as it pertains to specific applications increases the user's ability to compare antibody ratings.

To encourage comprehensive adoption of these guidelines, journal editors, funders, producers, and users should work together to increase transparency of antibody and validation information, develop systems for monitoring and communicating information about product quality and consistency, and evaluate new scientific information and technologies for their utility in validating antibodies. There was consensus that a scoring system may be a better way to achieve improvement in antibody quality. GBSI is initiating working groups to formulate a set of application-specific standards combined with a scoring system (a "GBSI score") based on validation information from the literature and concepts discussed at the Workshop.

Addressing the irreproducibility challenge associated with antibodies is a community-effort, with shared responsibility among all interested stakeholders, regardless of their sector. The diverse stakeholder community represented at the 2016 Asilomar Workshop on Antibody Validation: Standards, Policies, and Practices is committed to leading the charge for widespread adoption through practice, training, and certification. The Workshop participant list will be used in the future for communication and consensus testing as the validation scoring system evolves and is established.

APPENDIX B:

Table of Required or Requested Antibody Information

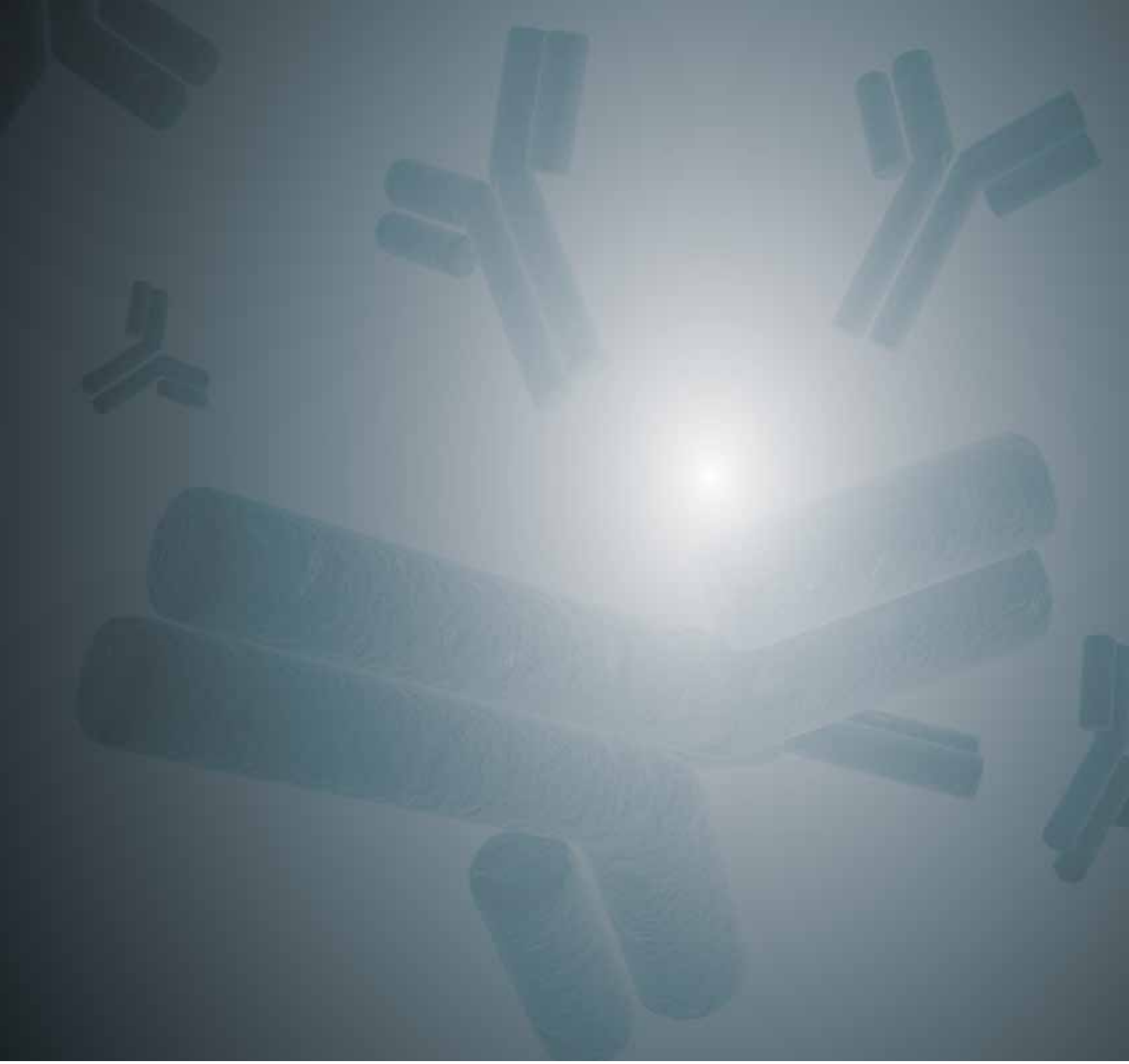
The table lists the antibody-related information required or requested by 19 publishers and other organizations.

Journal/ Organization	Unigue Materials Availability/Identification	RRID Participation	Ab Validation Requirements	Validation Requirement Strictness*
1	Yes	Required	Yes	In-depth
2	Yes	Required	Yes	In-depth
3	Yes	No	Yes	In-depth
4	Yes	No	Yes	In-depth
5	Yes	Encouraged	Yes	Intermediate
6	Yes	No	Yes	Intermediate
7	Yes	No	Yes	Intermediate
8	Yes	No	Yes	Intermediate
9	Yes	Encouraged	No	Minor
10	Yes	Encouraged	No	Minor
11	Yes	Encouraged	No	Minor
12	Yes	Encouraged	No	Minor
13	Yes	Encouraged	No	Minor
14	Yes	No	No	Minor
15	Yes	No	No	Minor
16	Yes	No	No	Minor
17	Yes	No	No	Minor
18	Yes	No	No	Minor
19		No	Yes	Intermediate

*In-depth - Identifies specific validation requirements / guidelines

Intermediate - Provides vague validation or other requirements

#AbValidate



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