The Pivotal Role of Producers and Service Providers

The following Consensus Building Paper presents key topics described in the following sources: comments from pre-workshop online discussions made on protocols.io; feedback from the Workshop Steering Committee; and relevant publications. All content not referenced is derived from online discussions. Source material as of August 30, 2016.

The purpose of this paper is to provide a starting off point for the dialogues and consensus-building process at the *Antibody Validation: Standards, Policies, and Practices* Workshop. The conclusions and preliminary recommendations are not final but are meant to serve as a basis for further discussion.

The $1.6 billion dollar research antibody market is very large, containing 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, resulting in irreproducible results and lost resources (Berglund et al, 2008; Bradbury & Pluckthun, 2015a; Bradbury & Pluckthun, 2015b; Egelhofer et al, 2011; Michel et al, 2009). The lack of defined standards used to characterize and validate antibodies is a key contributor to this problem. However, the economics driving the antibody market, abundance of available reagents, and existing practices by antibody manufacturers and vendors further complicate the matter (Marx, 2013). The concepts, perspectives, and preliminary recommendations described herein are derived from the pre-workshop online dialogues and published literature. The recommendations are intended to promote thoughtful consideration of antibody validation practices and challenges, the roles and responsibilities of users and producers, the role of certification, and the impact of service providers supporting antibody validation. This summary paper will serve as a basis for building consensus around these issues at the Antibody Validation: Standards, Policies, and Practices Workshop.

QA/QC and Antibody Validation by Producers – *Antibody Validation Standards* | Antibody manufacturers use a range of QA/QC and validation processes (Figure 1). Select companies carry out in-depth validation of reagents and provide extensive information on antibody properties, validation strategies, and experimental conditions for testing (Baker, 2015a). A review of validation practices across the industry highlight two central problems (Bordeaux et al, 2010):

1. Lack of consistency in QA/QC and use of controls;
2. Limited information-sharing enabling the evaluation of existing validation data.

These challenges lead to many research antibodies being unsuitable as binding reagents due to sub-par sensitivity and cross-reactivity with other substrates, a feature rated as one of the biggest difficulties with reproducibility.

Tier 3 | Minor

- Recommended applications and dilutions
- Animal host
- Immunogen type (e.g., peptide or recombinant protein)

Tier 2 | Intermediate

- Immunogen sequence or region
- Validation data for at least one of the recommended applications
- Genetic strategies (over-expression system)
- Peptide blocking

Tier 1 | In-depth

- Detailed validation information
- Application of multiple validation strategies
- Testing across multiple cell lines
- Genetic strategies
- Testing across multiple applications
- Detailed SOPs
- Reproducibility
- Test batch-batch consistency

Figure 1 Antibody validation practices of companies: Bordeaux et al. evaluated seven companies for their antibody validation practices and categorized three tiers from minor to in-depth validation based on information provided (Bordeaux et al, 2010).

Antibody Validation
Standards, Policies, and Practices

GBSI
Global Biological Standards Institute

Reproducibility | Lot-to-lot variability of commercial antibodies is a substantial challenge (rated as the top problem with research antibodies by 40% of respondents in a Twitter poll on protocols.io). Variability in antibody performance is, in part, tied to their underlying biology. Even when using the same immunogen, batches of polyclonal antibodies differ from animal to animal, and hybridomas can drift over time leading to changes in produced antibodies (Baker, 2015a; Bradbury & Pluckthun, 2015b). Similarly, changes in expression conditions for recombinant antibodies can ultimately affect performance. To control for variability in antibody production, two scenarios apply:

- Validation of every new batch of antibody using the same applications and experimental conditions to demonstrate consistency and reproducibility;
- Pooling sera after positive screening to reduce the variability among polyclonal antibody batches

Reagent Identification | A lack of transparency, traceability, and the use of identical catalog numbers across batches and use of inconsistent reagent names across suppliers also are problematic (Marx, 2013)(Voskuil, 2014; Weller, 2016). Many vendors sell antibodies from other producers through external manufacturer (OEM) agreements (Voskuil, 2014). Some vendors will change catalog numbers obscuring the traceability of the reagent in accordance with these agreements. In fact, through these agreements, manufacturers often are not contractually permitted to reveal the source of an antibody. Rebranded antibodies provide many companies with increased revenue, marketing, and budgets. In addition, some companies will replace one stockpile with another from a different manufacturer without notice if they determine that the two reagents are biosimilar. As with reagent names, the use of consistent validation data also varies across companies. Furthermore, validation data may come from the original manufacturer or the reseller. While the re-validation of antibodies is supported by some contributors on the online dialogues, it further obscures redundancy in the market if the reagent name is not maintained. Possible solutions to this problem include:

- A distinct catalog number applied to each new batch of antibody. Successive versions of the previous catalog number can be used to maintain continuity (e.g., two separate batches of the antibody labeled distinctly as 1584-A and 1584-B).
- Consistent identification of catalog numbers across manufacturers and suppliers during distribution. This possible solution would prevent the propagation of low-quality reagents by reducing the commercial market for these antibodies. Furthermore, this solution would ensure that researchers could consistently identify antibodies when repeating studies.

Roles and Responsibilities of Researchers, Producers, and Suppliers | The antibody market is complex, wherein the key players (i.e., manufacturers, resellers, aggregators, and distributors) may play specific roles in promoting antibody validation based on the service they provide. Manufacturers produce and sometimes sell antibodies, while suppliers re-sell antibodies from other companies with and without re-branding. Some companies produce antibodies in-house and re-distribute. Aggregators have limited customer facing services; their primary function is to collect products from small companies for distribution. Distributors provide access to reagents and expand reach. The research antibody industry, as a whole, has the responsibility of providing their customers with quality reagents that consistently function as described. This includes providing researchers with complete information about the reagent, source, and validation data and with affordable customized antibody panels, which would enable researchers to identify and validate reagents according to their scientific needs. Researchers also share responsibility for appropriate selection and use of antibodies in research, including adequately validating antibodies and reporting of reagent performance (Helsby et al, 2013; Voskuil, 2014). Suggested roles and responsibilities of antibody producers and users are shown (Figure 2). Key economic challenges for antibody producers are listed (Figure 3).

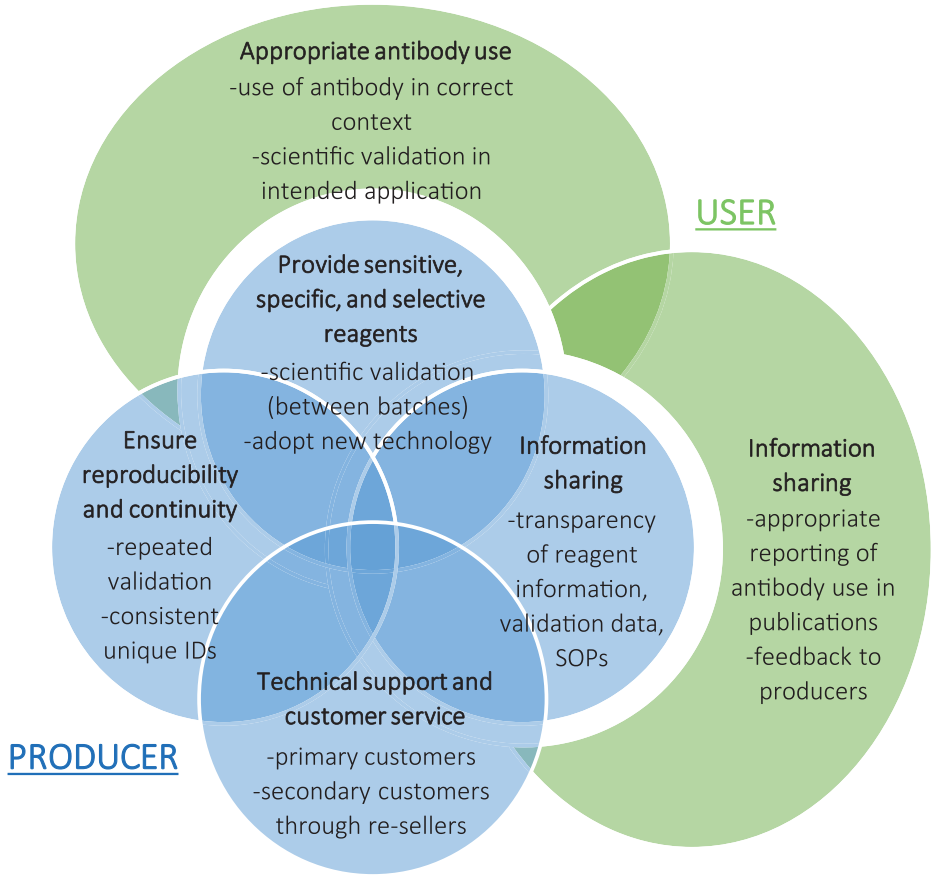

Figure 2 Roles of users and producers in antibody-based research.

Economic Challenges Facing Research Antibody Producers and Vendors

- Financial burden of stringent validation; across all applications; across an entire catalog
- Loss of competitive advantage with sharing peptide and antibody sequence information
- Complex system that relies on sharing of products among companies for increased revenues, marketing, and distribution
- Transparency of original manufacturer information in downstream sales

Figure 3 Summarizes the economic challenges of the high-volume antibody market felt by producers and vendors.

Service Provider-based Validation | Antibody validation can be time-consuming and cost-prohibitive. The use of third-party service providers allows for identification of high-quality reagents with transparent in-depth testing.

Certification to Strengthen Validation | Certification of manufacturers for antibody production quality upon shared validation data (with validation conducted either in-house or by service providers) conveys robustness of the validation data and antibody quality to users, and allows for selection of high validity reagents.

Recommendations for Discussion | The preliminary recommendations are intended to be discussed further during the Workshop.

- Producers should validate antibodies with rigorous scientific standards, make validation data accessible to consumers, list accurate product specifications, and provide accessible and comprehensive customer support. At the same time, users should select the appropriate reagents for its experimental use, validate antibodies in their intended experimental conditions (fit-for-purpose), and share results with the scientific community.
- Producers should list suitability of an antibody in the application in which it is tested successfully.

- Antibodies should be validated to ensure product consistency between batches. Polyclonal antibodies, in particular, must be validated for every batch. Antibody validation data should be kept up-to-date across all vendors.
- Producers should consider custom antibody panels that allow cost-effective testing of multiple antibodies by end-users.
- Antibodies should be assigned unique identifiers that indicate continuity between batches.
- Re-suppliers should validate antibodies independently from the original manufacturer, if possible. This includes validating antibodies between batches. However, not all companies have the laboratory capacity available to achieve this. In this instance, providing transparent information on the source of the antibody is beneficial.
- If possible, antibodies distributed by different manufacturers and vendors should use the same identifiers or provide transparent information on the source of the antibody. This may not be feasible given the structure of the research antibody market.
- Service providers can support effective antibody validation, while certification can be used to indicate which producers develop high-quality reagents.

Concluding Remarks | Producers play a pivotal role in setting the minimum level of quality for available affinity reagents, while service providers supplement antibody validation processes by providing third-party evaluation of antibody quality. Defensible science, transparency, and consistency of product and identifiers will enhance research reproducibility.

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For further information:
202.772.0133
info@gbsi.org
www.gbsi.org
Follow us on Twitter @GBSIorg

GBSI Global Biological Standards Institute

1020 19th St., NW, Suite 550
Washington, DC 20036 USA