The AMERICAN ASSOCIATION of IMMUNOLOGISTS

Response of The American Association of Immunologists (AAI) to the NIH Request for Information: Inviting Comments and Suggestions on the Reagent-Related Barriers to Reproducible Research
(http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-020.html)

Submitted on behalf of AAI by Clifford V. Harding, M.D., Ph.D., Chair, AAI Committee on Public Affairs

The American Association of Immunologists (AAI), the largest professional association of immunologists in the world, representing more than 7,600 basic and clinical immunologists, appreciates this opportunity to submit comments to the National Institutes of Health (NIH) "Request for Information (RFI): Inviting Comments and Suggestions on the Reagent-Related Barriers to Reproducible Research" (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-020.html).

Reproducibility in preclinical research is important for the advancement of basic research and for the translation of these findings to potential therapeutics. In this RFI, NIH acknowledges that "[m]any factors influence reproducibility in biomedical research…." Because "the role reagents play in the reproducibility of research" has been a "consistent concern" of stakeholders, and because variations in reagents "have the potential to yield dramatic differences in the resulting data and conclusions[,]" NIH has issued this RFI in an effort "to understand and consider the various reagent-related challenges facing the research community."

As NIH considers how to address these challenges, AAI urges NIH to recognize that complex biological processes can themselves lead to variability in results, which is not inherently negative for the scientific community. Conflicting findings can be beneficial to research by spurring further investigation, which may result in a more detailed elucidation of a particular question. In addition, NIH should examine whether having all information that *seems* useful would actually result in better reproducibility. Finally, AAI recommends that NIH avoid implementing requirements that may lead to additional regulatory burden.

Despite these caveats, AAI agrees that irreproducibility due to variation in documentation, usage or handling of reagents can lead to lost productivity and poorly utilized funds. AAI therefore proposes the following to reduce the likelihood that reagents would be a barrier to reproducibility.

9650 ROCKVILLE PIKE, BETHESDA, MARYLAND 20814-3994
PHONE: 301-634-7178 * FAX: 301-634-7887
E-MAIL: INFOAAI@AAI.ORG * WEB: WWW.AAI.ORG

Comment 1

Experiences regarding variation in reagents/resources, including:

- **Barriers to identifying reagent composition and methods used to prepare, process, or store specific reagents**
- **Documentation accompanying proprietary manufacturer-prepared kits**
- **Characterization and/or validation of reagents/resources**
- **Batch effects and variability of the same reagents' performance between different labs and with storage time**

As mentioned in the RFI, lack of documentation by both reagent companies and researchers can result in variability in research findings. In most instances, however, these companies, rather than the researchers, control how reagents are manufactured, handled, and transported, and determine what information is provided to investigators. More information is often required from the companies in order to determine the composition and comparability of similar reagents from different companies. This issue could be addressed in part by requiring the companies to disclose more information, for example, the presence of stabilizing or preservative agents in the product, the type of product validation performed, and a clear "use-by" date on the reagent. Further, the companies should reveal more detail regarding the exact protocol(s) and dilution(s) used for product validation.

Comment 2

Solutions to the following issues:

- **Common problems with reagents and techniques for developing and storing reagents**
- **Needs for improved reagents or techniques for developing reagents, including the role of standard protocols**
- **Actual or perceived barriers to improvements in reagent quality and accessibility**
- **Needs for standardized terminology**

Researchers will often use several different products in an effort to best optimize their assay. Some come across products that fail to yield the advertised results. It would greatly benefit the scientific community if these specific failures were publicly disclosed. This information would save researchers time and money by alerting them to potential issues with that product. It would also make companies more aware of these failures and provide an incentive and opportunity to address them. Therefore, it would be beneficial for companies to be encouraged to provide a public record of the feedback they receive on a specific reagent.

9650 ROCKVILLE PIKE, BETHESDA, MARYLAND 20814-3994
PHONE: 301-634-7178 * FAX: 301-634-7887
E-MAIL: INFOAAI@AAI.ORG * WEB: WWW.AAI.ORG

Additionally, some reagents are developed by researchers in-house. In some cases, there may be little quality control between lots or batches of reagents generated; this practice may lead to variability in results produced using reagents from different batches. To reduce this variability, NIH should encourage the use of Good Laboratory Practices (GLP) in laboratories that generate their own reagents.

Comment 3

The reagents, techniques, and tools used to improve reagent reproducibility and consistency, including barriers to use.

Comment 4

The means by which students become trained in the consideration of reagent variability as a source of experimental irreproducibility and the processes to control it.

Prior to starting significant studies in the laboratory, students should be trained on proper record keeping for reagents and on the process of optimizing assays using similar reagents from varying sources.

Comment 5

Best practices for chain of custody procedures, such as how reagents are handled, including packaging and temperature control during shipping, and stored from manufacture through use

See answer to Comment 1

Comment 6

Suggestions about best practices for sharing information, including:

- **Reporting of reagent or resource identification in publications**
- **Changes in quality/activity of reagents**

The methods section of any published manuscript is critical for determining why results among laboratories may differ. NIH should urge authors to include in their methods sections applicable details for each reagent used, including but not limited to:

- commercial reagents: manufacturer, catalog number, lot number, concentration used, dilution buffer used, storage conditions;
- animals: breeding facility, age at use, gender used, weight at use, infection status, number of backcrosses performed on transgenic or knock out mice, source and treatment of water, type of chow used in the facility, conditions of animal facility;

- cell culture: passage number (if known or acknowledged if not), time in culture, date of last mycoplasma testing, derivation of each cell line (or source location), any selection performed, genetic confirmation of line, serum source (including stock number, lot number, and whether it is heat-inactivated);
- primary human cells: gender, any genetic analysis, associated annotation, source of the cells or tissue used; and
- quantitative methods: number of times experiment performed, number of samples used per group, specific method of quantification, details of statistical analyses performed (and which groups were compared).

Another issue that often complicates reagent reproducibility is the referencing of publications in the methods section of articles. If authors use a protocol that is similar to one that has previously been published, the author will often write, "…as described elsewhere [citation]," rather than rewriting the protocol. To ease the burden of page restrictions on journals and researchers, AAI suggests that the NIH consider creating a repository for detailed protocols that could be referenced in publications.

9650 ROCKVILLE PIKE, BETHESDA, MARYLAND 20814-3994
PHONE: 301-634-7178 * FAX: 301-634-7887
E-MAIL: INFOAAI@AAI.ORG * WEB: WWW.AAI.ORG