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Assay Guidance Manual for Drug Discovery: Robust or Go Bust

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There have been considerable efforts in the last two decades within the biomedical science community to address the crisis of irreproducibility in basic and preclinical research.[1] Robust assays, with rigorous data analysis reporting standards, help to prevent irreproducibility. Every successful drug discovery campaign begins with the right assay —an assay that measures a biological process in a physiologically relevant and robust manner. Although assays vary substantially with respect to design, speed, throughput, and complexity, they must be robust for an effective drug discovery campaign. This special issue is focused on the Assay Guidance Workshops for High-throughput Screening and Lead Discovery conducted by the *Assay Guidance Manual* (AGM) program of the National Center for Advancing Translational Sciences (NCATS).[2] The articles expand on workshop lecture concepts by incorporating best practices in assay methodologies to enable reproducible results and illustrating how these principles are critical to the entire drug development process. The AGM program is part of a disease-agnostic translational science education program that helps bridge the gap between discoveries and the delivery of new therapies by establishing and disseminating standards for rigor in early translational research. As a component of this program, the AGM[3] is a free and publicly available e-book of best practices for the design, development, and implementation of robust assays in preclinical research. The workshops, conducted by AGM editorial board members, authors, and other subject matter experts, highlight key concepts from the AGM. The goal of the AGM workshop series is to improve the design, rigor, and execution of assays supporting preclinical discovery by providing participants with a broad, practical perspective on assay development and data analysis.

This special issue of *SLAS Discovery* contains 10 peer-reviewed articles, including 7 original research articles and 3 perspectives. Most articles were written by speakers of the AGM workshop series, members of the AGM's editorial board, and authors of the manual. The articles span a wide range of topics within the scope of the AGM workshop series and can generally be grouped into the following topic areas: (1) best practices in assay development, data analysis, and implementation for high-throughput screening (HTS)

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and lead discovery; (2) assay development and validation; and (3) the utility of specialized assays in lead discovery and target validation.

The first topic area has taken center stage in this special issue and contains five articles (three perspectives and two original research articles) by experts from academia, government, and industry that are focused on best practices in assay development, data analysis, and implementation for HTS and lead discovery. In the first perspective, Born et al.[4] provide the medicinal chemist's viewpoint on the importance of assay design for successful chemistry efforts. By providing case studies from both NCATS's division of preclinical innovation and the literature, the team not only highlights the critical role of assay design, but also opens a much-needed dialogue between biologists and chemists. The other two perspectives emphasize the importance of following proper guidelines in cell culture practices. Riss et al.[5] give a broad overview of good cell culture practices and standard operating procedures for handling cultures, while Korch and Capes-Davis[6] highlight the problem of cell line misidentification and its enormous detrimental impact if left undetected. Both perspectives provide tools and resources for readers to address this issue. The remaining two original research articles provide best practices for identifying compound interferences in biochemical assays (Coussens et al.[7]) and guidance for the use of robust statistical methods for the analysis of bioassay data as an alternative to standard methods (Haelewyn et al.[8]).

The second general topic area of this special issue includes three original research articles that cover in vitro assay development and validation as well as validation of predictive in silico models for ADME (absorption, distribution, metabolism, excretion). Kaur et al.[9] present the development, optimization, and validation of a novel complex tumor spheroid assay for HTS. This article provides a prime example of the steps needed to properly develop and validate 3D tumor spheroid models for pancreatic cancer, which can also be adapted to model other solid tumor types. In contrast, Wen et al.[10] highlight lessons learned from a failed assay development campaign to discover small molecules that can rescue radiation damage. This article is valuable as it demonstrates what assay developers encounter in the lab frequently—even with good practices, extensive efforts, and a strong rationale, scientists cannot always generate a robust assay for screening purposes. The final article in this topic area is by Siramshetty et al.[11] from NCATS. In this article, the authors update quantitative structure–activity relationship models for Tier I ADME assays used at NCATS and validate their performance against a set of marketed drugs. The authors also provide models and data sets as a publicly available resource to the drug discovery community (ADME@NCATS web portal).

The last topic area contains articles that focus on the utility of specialized assays in lead discovery and target validation. Lowell et al.[12] use a powerful combined transcriptomic-phenotypic screening strategy to identify novel transcriptional regulators of neurite outgrowth downstream of a multitarget kinase inhibitor. The second article in this category by Li et al.[13] describes the utility of a butyrylcholinesterase (BChE) enzyme-based inhibition assay for a quantitative high-throughput screen of nearly 9000 chemical compounds to identify potential BChE inhibitors rapidly and efficiently.

We wish to thank the authors who contributed and the SLAS staff and editors who helped assemble this special issue. There is a strong need to continue an open dialogue regarding best practices in preclinical research to avoid irreproducible results, increase efficiency in drug discovery, and save billions of dollars on research. To that end, we hope that this special issue helps scientists to recognize the importance of rigor in design, development, and implementation of robust assays. We also hope that this special issue elucidates powerful new methodologies and platforms for successful future drug discovery campaigns.

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Related Links

1. NCATS AGM program: https://ncats.nih.gov/expertise/preclinical/agm.
2. Assay Guidance Manual, National Library of Medicine (NLM), National Center for Biotechnology Information (NCBI) Bookshelf: https://www.ncbi.nlm.nih.gov/books/NBK53196/.
3. Assay Guidance Workshops for High-throughput Screening and Lead Discovery: https://ncats.nih.gov/events/Assay-Guidance-Workshop-for-High-Throughput-Screening-and-Lead-Discovery.
4. ADME@NCATS web portal: https://opendata.ncats.nih.gov/adme/.