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Six tips for effective assay validation for CGT with focus on flow cytometry

We asked experts with industry and regulatory background to share their insights on how to maneuver cell and gene therapy (CGT) assay validation throughout the product development lifecycle. The following list contains the main tips taken from their roundtable discussion.

1. Navigate validation with established guidelines

- For comprehensive and globally applicable guidance, refer to ICH Q2 and Q14 throughout the validation process.
- Additionally, for flow cytometry–specific protocols, refer to CLSI H62. This guidance document focuses on clinical laboratory diagnostics, with fundamental principles applicable to in-process controls and quality control (QC).

2. Understand your assay inside and out

The development phase is frequently rushed, but commercial readiness demands a deep understanding of analytics.

- It is crucial to consider what attribute an assay is assessing, and which performance parameters, such as precision, accuracy, linearity, and sensitivity are necessary.
- Utilize tools such as Quality Target Product Profile (QTPP) and Analytical Target Profile (ATP). These tools guide you toward a comprehensive understanding of your product's analytical requirements, ensuring readiness for commercial deployment.

3. Reduce operator variability for consistent results

Flow assays are likely to be subject to operator variability, introducing potential bias in sample preparation, data acquisition, and analysis.

- Mitigate human bias by implementing standardized protocols, automated procedures, and rigorous training programs.
- Emphasize consistency and adherence to protocols to minimize variability and ensure reliable results.

4. Harness collaboration for validation success

Assay validation is a collaborative endeavor that benefits from diverse expertise and perspectives.

- Ensure effective communication, cooperation, and coordination among process development, analytical development, MSAT, and QC personnel.
- Assign clear roles and responsibilities, encourage an environment of open dialogue with clear terminology, and leverage the collective knowledge and skills of the team to achieve robust validation outcomes.

5. Consider strategic timing

In CGT, where expedited regulatory pathways are prevalent, strategic timing in assay validation is paramount. It is advisable to start with the validation of safety assays and follow up with efficacy assays. Additionally, adopting a phased approach to assay development is key.

- Focus on generating comprehensive data early on to grasp both product and assay nuances, utilizing large multicolor panels.
- Reserve full validation efforts for markers crucial to ensuring product quality and safety.

Significance and reliability should be carefully weighed for every assay. While all release tests require thorough validation, assays designated for information only purposes may suffice for qualification.

6. Maximize workflow standardization, scalability, and compliance

Consider instrumentation, software, and reagents as interconnected components to optimize your flow cytometry workflow. By integrating these seamlessly, you can standardize procedures, enhance assay performance, and ensure regulatory compliance and scalability.

- Reagents: Choose recombinantly engineered antibodies for heightened consistency and specificity compared to traditional hybridoma-derived alternatives (e.g. REAfinity® Recombinant Antibodies).
- Instrumentation: Ensure harmonized instruments to facilitate seamless assay transfer. Compatibility with automation systems enables scalability. Additionally, automated compensation and sampling features and Levey-Jennings/daily QC functionalities enhance assay reliability (e.g. MACSQuant® Analyzer 16).
- Software: Select software enabling compliance with 21 CFR Part 11 regulations, enabling meticulous process documentation, user role management, and adherence to ALCOA++ principles (e.g. MACSQuantify™).
- Data analysis: Employ automated gating algorithms for streamlined analysis to reduce human bias and ensure robust data interpretation (e.g. Express modes)

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