

Developing Flow Cytometry Assays for ATMPs: A Best Practice Guide for the Cell and Gene Therapy Community

Identify assay purpose

- Starting material characterisation
- In process control
- QC batch release
- Identity / purity / impurity / other
- Broader for information only characterisation
- Clinical samples

System selection

- Laser configuration
- Availability of equipment across CROs etc
- Continuity of supply of reagents etc.
- Qualification (IQ/PQ/OQ)
- Platform end of life migration
- Comparability between systems – such as sensitivity

Sample knowledge

- Starting / process material variability
 - patient to patient
 - healthy vs diseased
 - media / buffers / excipients
 - prevalence of target cell population
- Method of sample acquisition
- Sample preparation effects
- Distribution / transport effects

Panel development / Staining protocol

- Purpose
 - Qualitative / quantitative / semi-quantitative
- Single staining versus multiplexing
- Raw material selection
 - Pre-mixes / lyophilised antibodies
 - Single / dual suppliers
- Reference standards
 - MESF
 - Positive / negative cell lines
 - Exemplar patient materials
 - TruCount beads

Data acquisition and analysis

- Pre-run calibrations
- Flow rates / voltages
- Compensation
- Threshold and debris exclusion criteria
- Gating strategy / automated gating
- Novel data processing strategies

Assay validation

- Strategy
 - Before or parallel to process validation
- Protocol development
- Requirements for dossier
 - How to present data
- Considerations for multi-site validation
 - Expected CVs between sites

Performance review & trending

- Data trending
- Operator training / experience / learnings
- Ongoing standards development
- Ongoing technology developments
 - High-throughput automation
 - New reference standards etc
- NEQAS
- Comparative data

Existing framework of regulation / standards / guidelines

- GMP
- ISO20387:2018
- ISO20391-1 /2: 2018
- ISO20688-1: 2019
- ISO/TS 20399-1/2/3
- USP<1027> / <127>
- Ph. Eur 2.7.24
- ISCT guidelines
- Others e.g. AABB

