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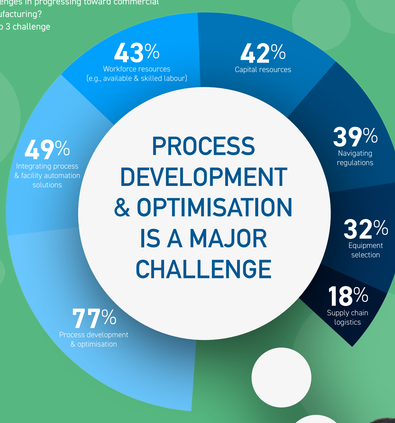
TOWARDS CONSISTENT CELL & GENE THERAPIES
- OVERVIEW OF THE ANALYTICAL TECHNIQUES NEEDED

77% of C&GT industry leaders worry that the challenges of process development and optimisation will prevent them from achieving commercial manufacture. Their concern is driven by process variability and uncertainty (55%) and regulatory issues (54%).

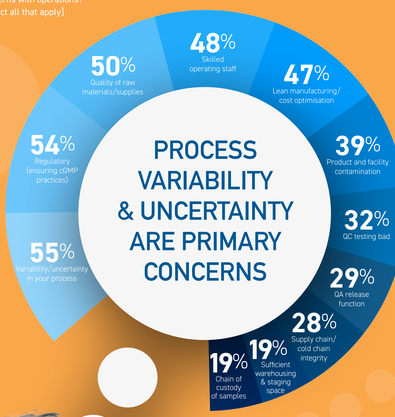
Q What do you consider to be your biggest challenges in progressing toward commercial manufacturing?
% Top 3 challenge



Most leaders in C&GT companies see process development & optimisation as a significant challenge in progressing towards commercial manufacturing.

*Source: CRB

Q What are your primary concerns with operations?
[Select all that apply]



Process variability or uncertainty and regulatory considerations are primary concerns for C&GT companies.

*Source: CRB

The C&GT industry demands new and better analytical tools to monitor and lower process development and product manufacturing variability. The chart below compares the analytical assays for assessing viral vector critical quality attributes (CQAs):

Current (blue) and next-generation (green) analytical assays for assesing viral vector CQAS.

Analytical methods for identity, quantity, and purity	Measurement	Pros	Cons	Suitability for C&GT Protein Analysis
SDS-PAGE	Protein identity Host Cell Protein (HPC) analysis	Low costs Fast	Low resolution Low sensitivity No info about PTMs	Low
Western Blot	Protein identity	Low costs High sensitivity	Low resolution No info about PTMs	Low
ELISA – Commercial	HCP analysis	Low costs Fast	Only analysis of a subset of HCPs	Low
ELISA – Process Specific	HCP analysis	Specific for the process	High cost Slow development Very difficult to use	Medium
HPLC	Capsid content analysis, full vs empty	Available in most analytical laboratories	Low resolution Low sensitivity No info about PTMs	Medium
PCR	Vector genome verification and titer	Robust, easy, and fast assay	Results affected by experimental factors	Medium
Optical Density	Viral titer	Simple, rapid, and direct method	Obscured by capsid impurities and aggregates	Medium
Analytical Ultracentrifugation	Empty vs full capsid titer	Reproducible, direct analysis, fast to develop	Complex, low throughput	Medium
Transmission Electron Microscopy (TEM)	Empty vs full capsid titer	High level of details for impurity analysis and capsid content	Complex, low throughput	Medium
Size Exclusion Chromatography - Multi Angle Light Scattering (SEC-MALS)	Vector characterization and quantification	Fast, accurate, and reproducible characterization	High level of expertise Expensive equipment Low throughput	Medium
LC-MS/MS	Protein identification & detailed characterization incl. mapping of PTMs HCP analysis	Very specific & informative Highly reproducible Can identify & quantify proteins from various sources & species in a single assay	Complex sample preparation High level of expertise Expensive equipment	High
RP-LC-MS	Protein identity, for capsid content analysis, full vs empty	Molecular Weight (MW) determination of individual viral proteins	Difficult to separate viral proteins Expensive equipment	High
IEX-LC-MS	Capsid content analysis, full vs empty	Detailed and exact information about charge variant of viral proteins	Complex Expensive equipment	High
Capillary Isoelectric Focusing (cIEF)	Capsid content analysis, full vs empty	Detailed and exact information about charge variant of viral proteins	Risk of protein aggregation blocking capillary	High

Highly reproducible analytical methods to monitor CQAs and ensure robust and consistent product manufacturing is still in development - but is essential for the future success of C&GTs.

Mass spectrometry combined with chromatographic methods have the potential to become such methods.

*Source: CRB Report [CRB Horizons. Cell and Gene Therapy Report, 2020/2021. Downloadable from https://go.crbgroup.com/2020-horizons-atmp]

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