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The next generation of AAV analytics

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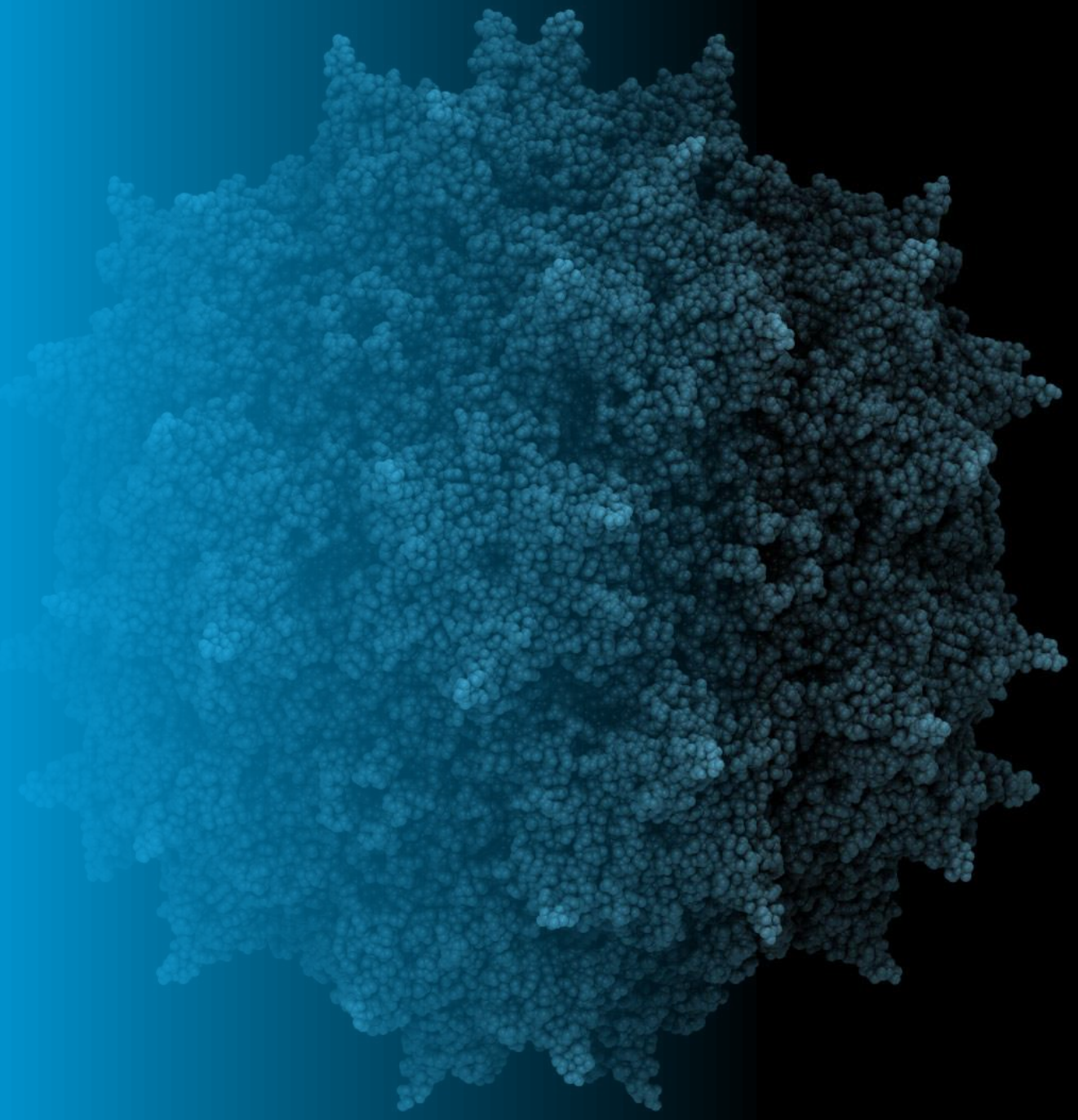
Senior Analytical Scientist

Development of AAV therapeutics
KTN - Cambridge 30 Jan 2019



Who we are

Part of a **world-leading network** of technology and innovation centres

Provide access to unique technical **facilities** and **expertise** to help adopt, develop and exploit innovations

Bridge the gap between businesses, academia, research and government

Were established by Innovate UK as a **not-for profit**, independent centre

It is our vision for the **UK** to be **a global leader** in the development, delivery and commercialisation of cell and gene therapies.

Where **businesses can start, grow and confidently develop** advanced therapies, delivering them to patients rapidly and effectively.

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Who we are

2012 | 2016 | 2017 | 2018 | 2019

Cell Therapy catapult Centre of excellence in innovation

Cell and gene therapy Catapult - CGT

Manufacturing centre

qPCR
ddPCR
DLS
HPCE
ELISA
MADLS
Western blot
Auto-mated WES
FACS
Infect-ious titre
LC-MS
Trans-ducing units
Synpromics
Sterili-ty
Impe-dence
Aggreg ation
Purity
Empty /full
Physi-cal titre
Ovisio
Raman
VCN
CGT Core projects

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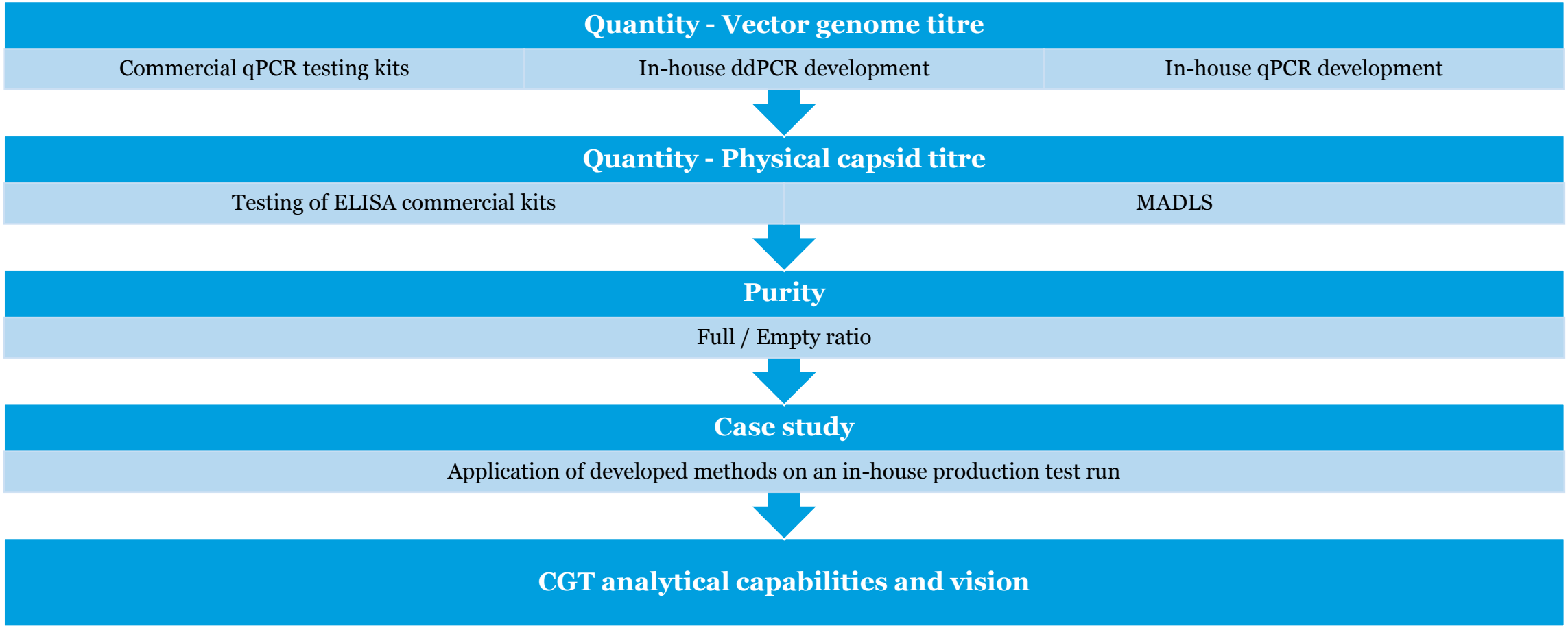
Presentation workflow



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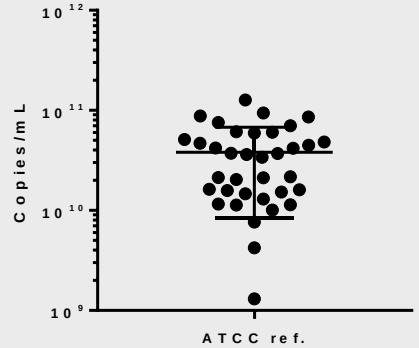
Quantity – vector genome measure

Quantity – vector genome titre

Traditional methods

Quantitative real-time PCR (qPCR)

- Primer and probes targeting gene of interest and/or ITRs
- Use of a digested plasmid as a standard curve



Limitations

1. Highly sensitive to PCR inhibitors – viral proteins and/or vector diluent → decrease in amplification efficiency → Under-estimation of viral titre
2. Bias from amplification efficiency – especially if targeting ITR region → under-estimation of viral titre
3. Bias introduced from the standard curve – amplification of dsDNA vs ssDNA → Over-estimation of viral titre
4. Steps above → High inter/intra-assay variability

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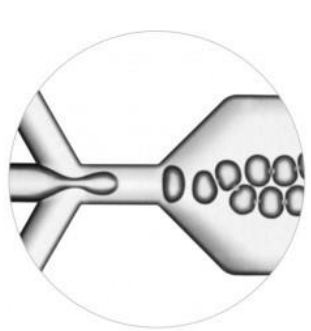
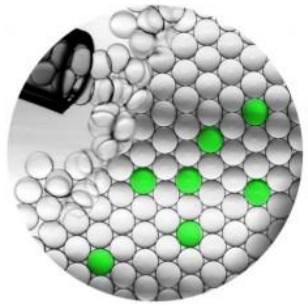
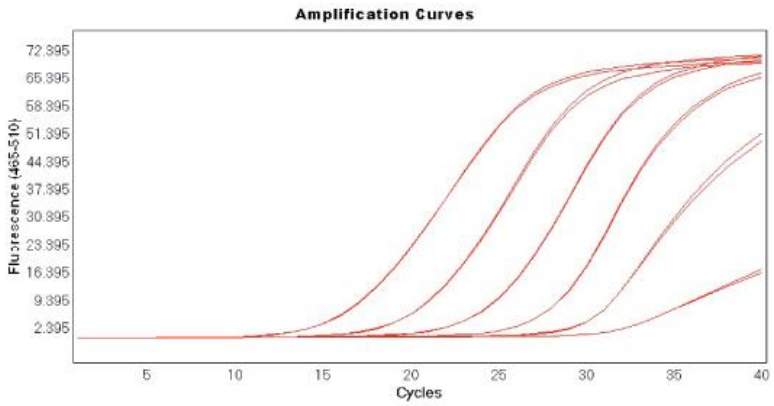
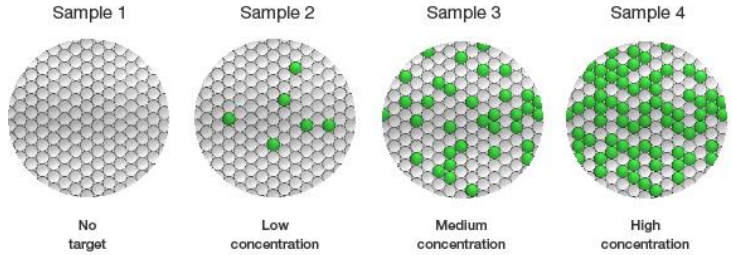
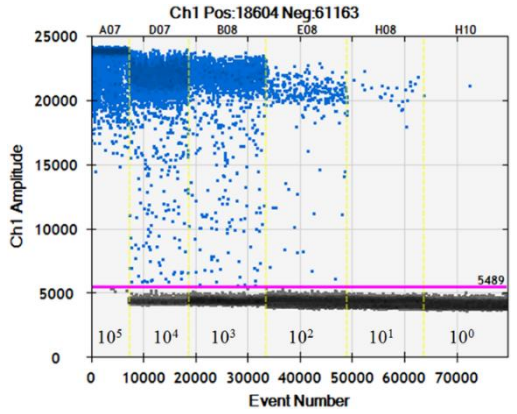
Quantity – vector genome titre

Aim
Develop a robust, accurate in-house method for measuring AAV2 vector genomes (and AAV2 derived serotypes)

qPCR alternatives - ddPCR

qPCR	ddPCR
	  
	 

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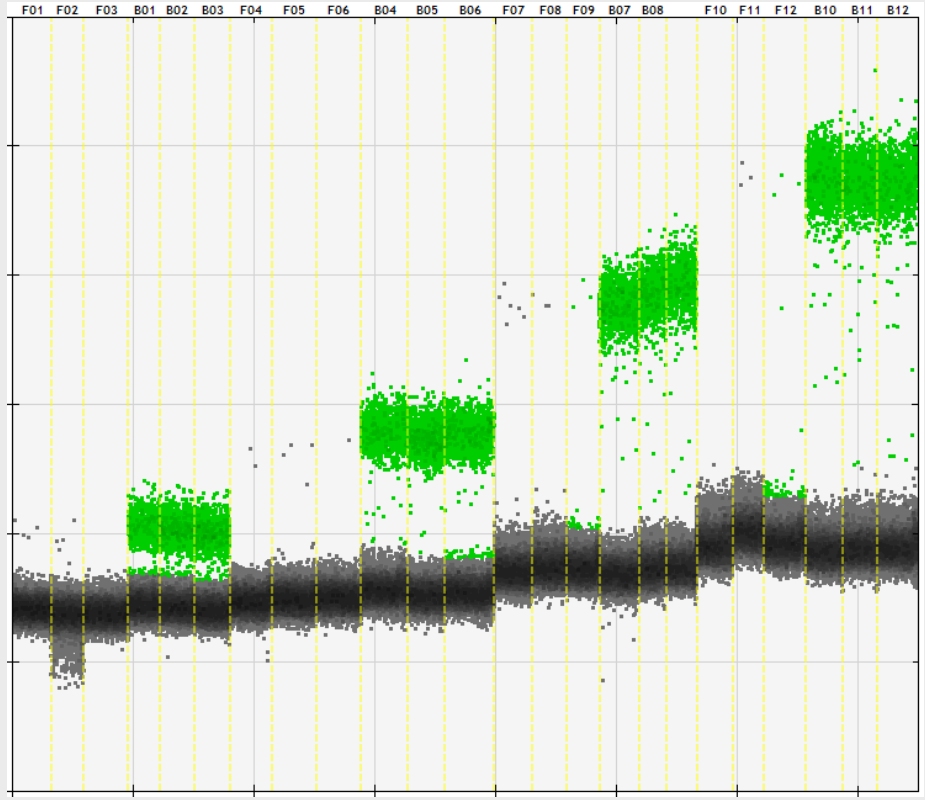
Scope

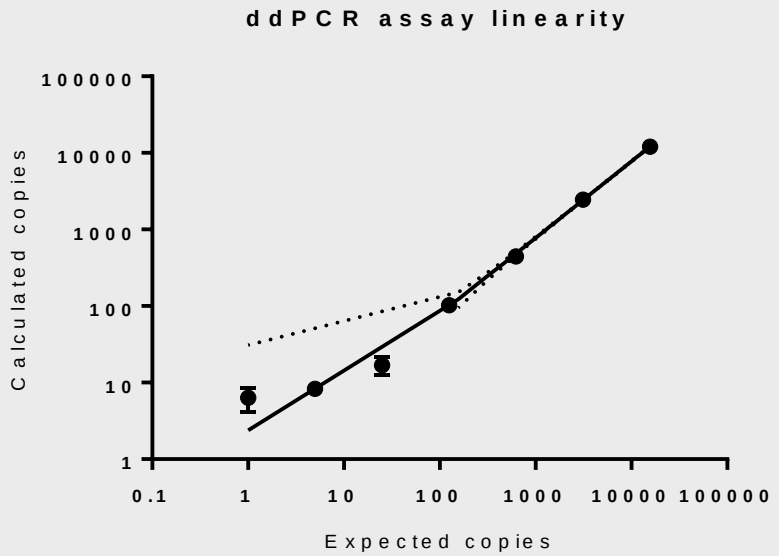
qPCR vs ddPCR

1. Highly sensitive to PCR inhibitors – viral proteins and/or vector diluent → decrease in amplification efficiency → Under-estimation of viral titre
   - ✓ Less sensitive to PCR inhibitors → suitable for in process vector genome measurement
2. Bias from amplification efficiency – especially if targeting ITR region → under-estimation of viral titre
   - ✓ End product measurement – less dependent on amplification efficiency → Suitable for targeting ITRs – Universal assay
3. Bias introduced from the standard curve – amplification of dsDNA vs ssDNA → Over-estimation of viral titre
   - ✓ Absolute quantification – no standard curve required → Improved precision
4. Steps above → High inter/intra-assay variability
   - ✓ Robust and accurate method for in-process control and product characterisation

ddPCR vg titre assay

Primer/probe set targeting ITR2 sequence – matching against internal positive control primer/probe set





R square	0.9969
Deviation from linearity	non significant
LLoD	16.16 copies
LLoQ	125 copies
Intra/inter assay CV	< 20%

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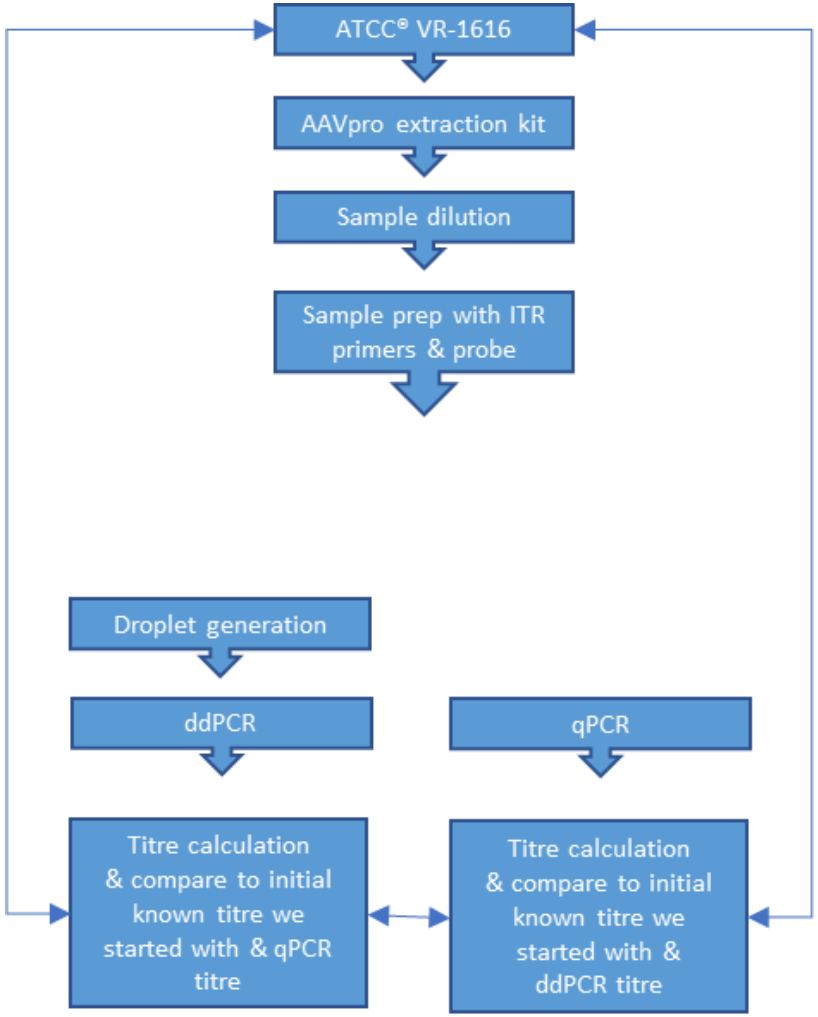
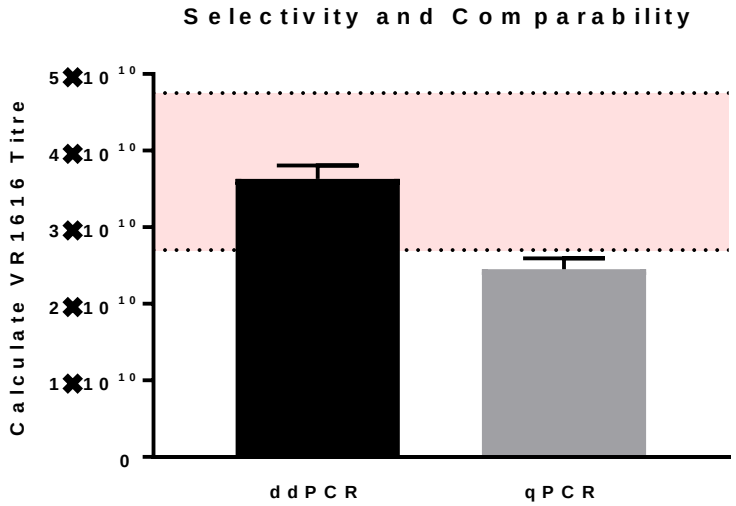
ddPCR and Commercial qPCR method comparability





ddPCR results within 95% CI

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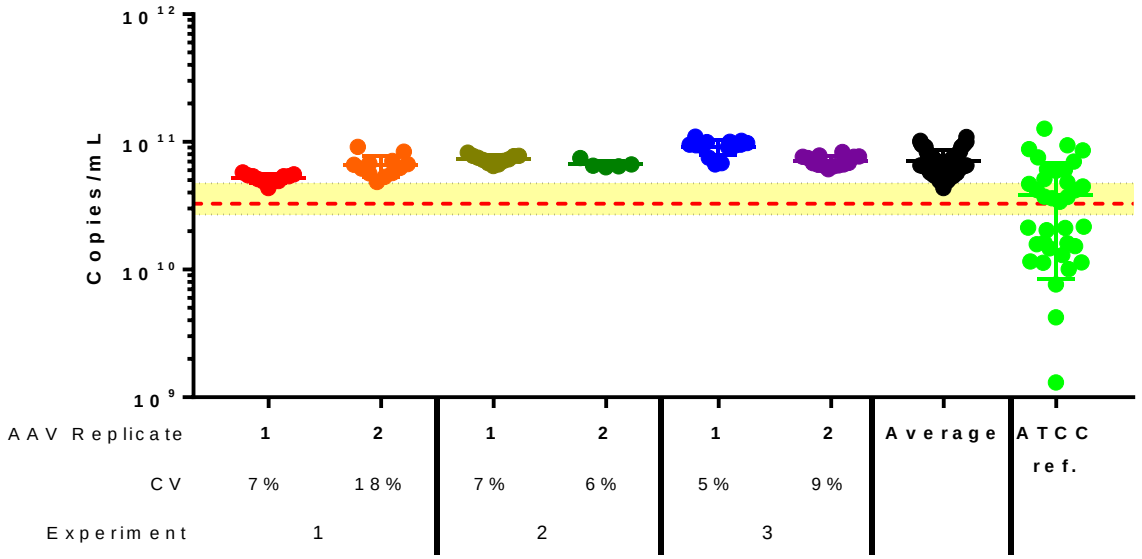
Summary

Fully functional assay offering

1. ITR sequence detection → Applicable to any AAV2 and AAV2 derived serotypes
2. In-house designed primers and extraction method
3. Higher sensitivity → Suitable for in-process sample measurement
4. Increased precision and reproducibility over commercial and current available qPCR titration methods

To overcome ddPCR limited market coverage, CGT has adapted in-house primers/probe set to a qPCR platform

Adapted in-house qPCR method



Intra-assay CV < 10%

Inter-assay CV = 22%

Inter assay variability greater than seen on a ddPCR platform, but still meeting acceptance criteria

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Quantity/purity
total particle measure
Empty to full ratio

Quantity/purity – total particle measure/ Empty to full ratio

ELISA – Commercial kit

Evaluation of 5 commercial kits

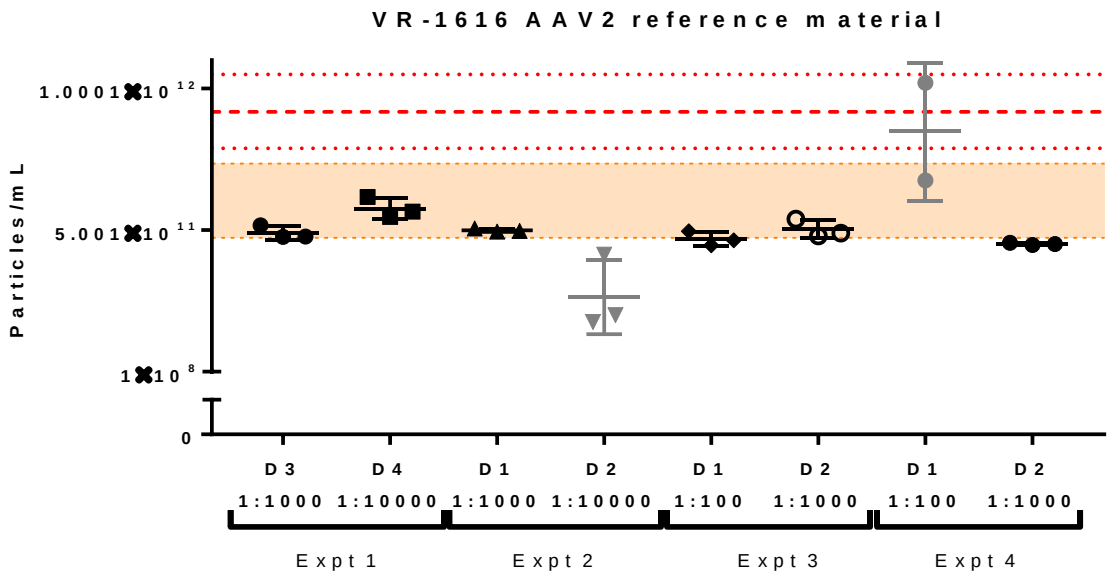
Inter assay CV <8%

Intra assay CV <5%

	qPCR	ddPCR
% full particles	13.69%	6.72%

Limitations

1. Expensive
2. Labour intensive
3. Time consuming
4. Antibody specific/serotype dependent



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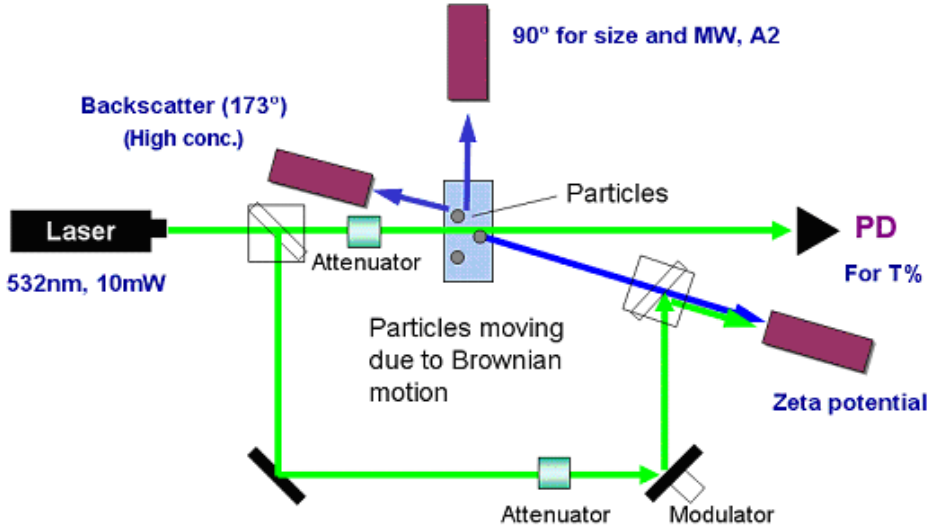
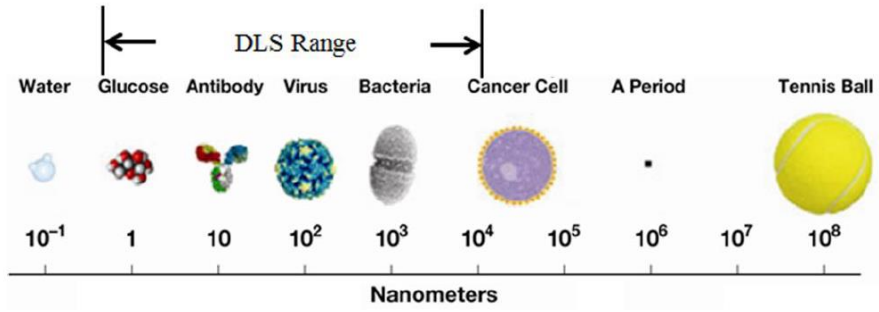
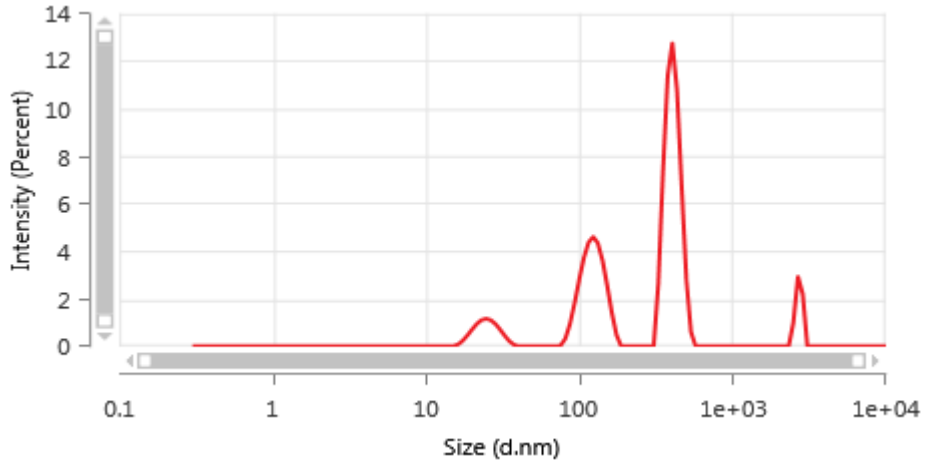
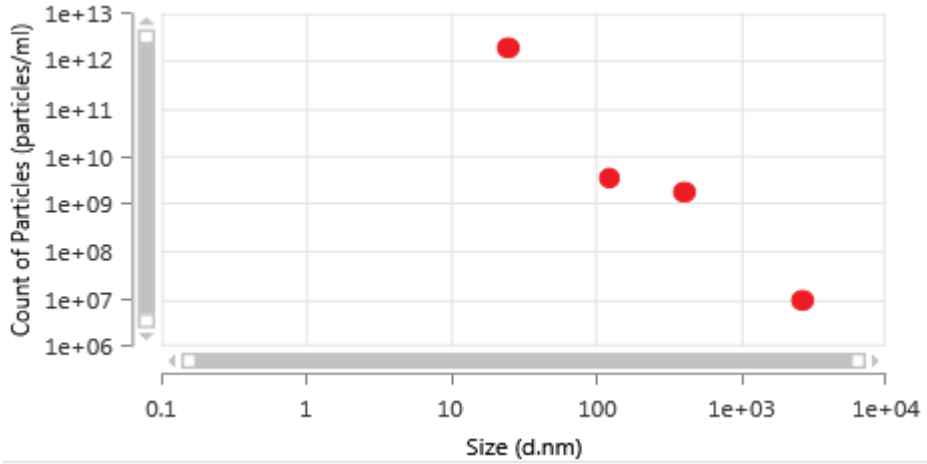
Dynamic Light Scattering technology









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Quantity – total particle number, alternative methods

MADLS and ELISA method comparability

1. Expensive
   - ✓ Long term cost efficient
2. Labour intensive
   - ✓ No sample prep required
3. Time consuming
   - ✓ Fast, readings in under a minute
4. Antibody specific/serotype dependent
   - ✓ Physical measure of particle content, universal serotype measure

- ✓ Measures impurities
- ✓ Measures aggregates

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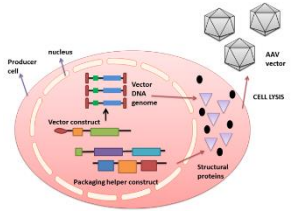
Case study

Overview of downstream developmental scope



Cell disruption
Viral vector release development and optimisation

Nuclease treatment
DNA removal development and optimisation

Clarification
Harvest filtration development

AKTA™ Avant
Chromatography purification development and optimisation

TFF KrosFlo UF/DF
Concentration & buffer exchange development and optimisation

Final filtration
Final filtration development

Cell lysis methods.

Source nucleases
Optimise treatment.

Cell lysis methods.
Filter options

Capture and polishing

Formulation concentration

Sterile filtration
Fill and Finish

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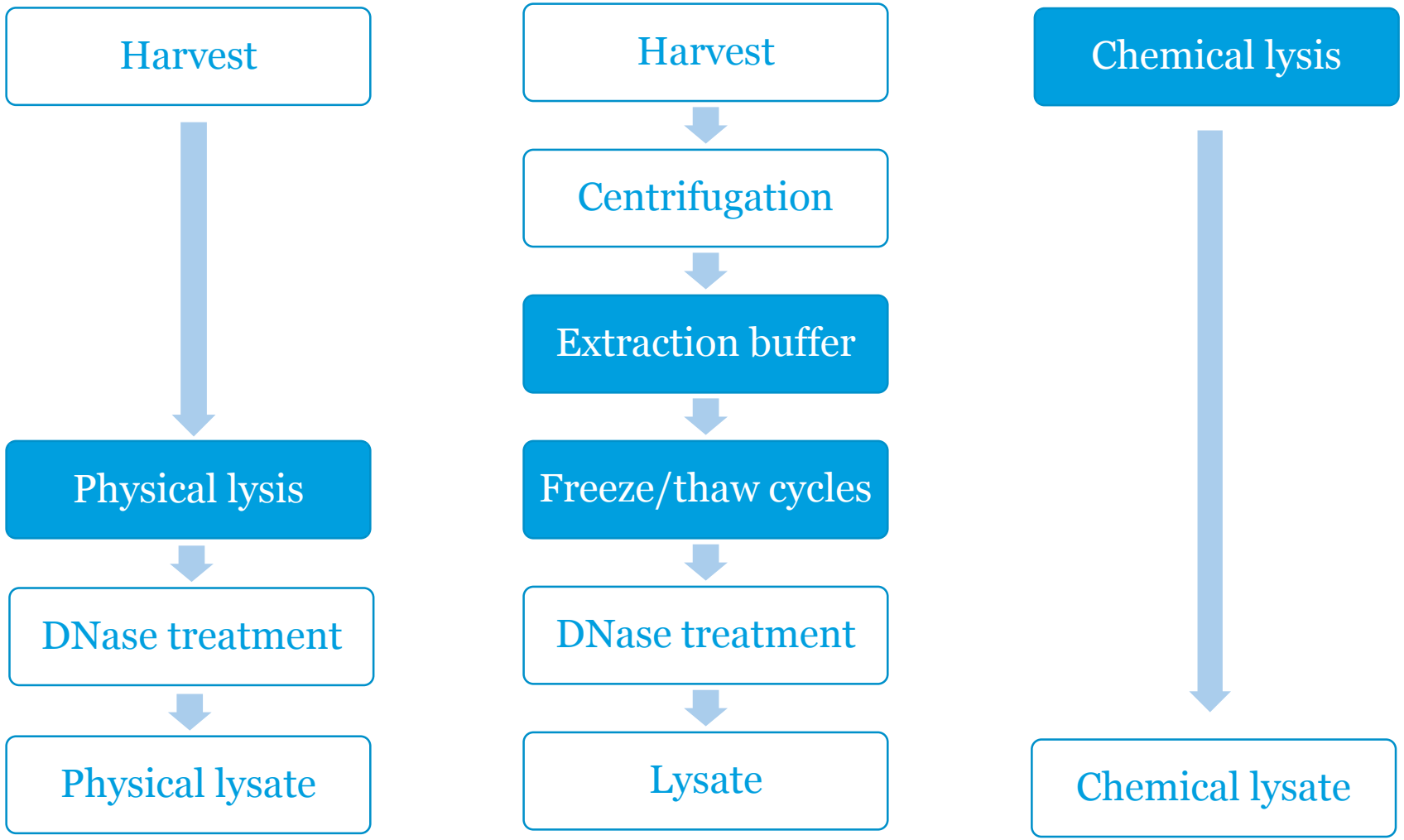
Cell Lysis Experiment – Study Design & Workflow



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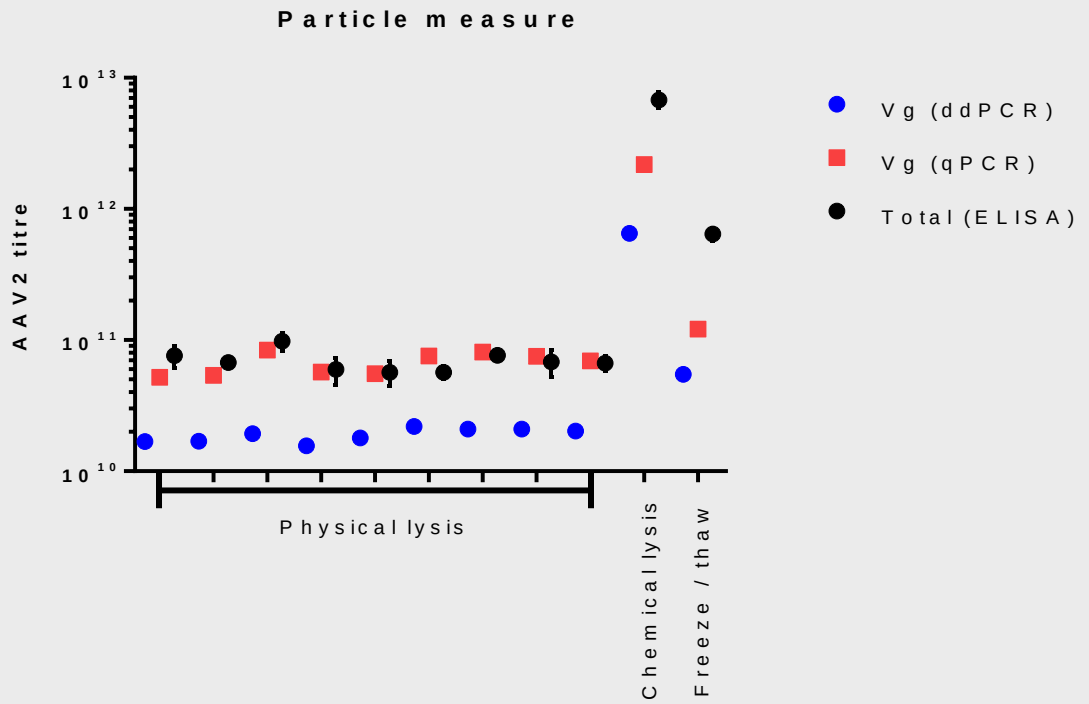
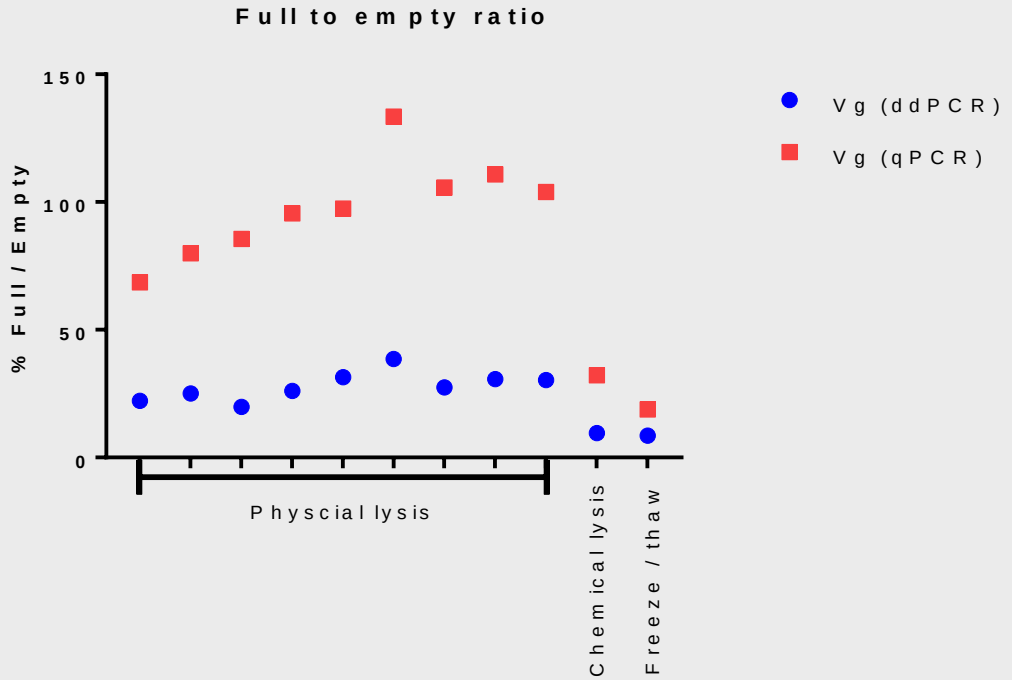
AAV titre and purity check

Particle number produced



Sample purity



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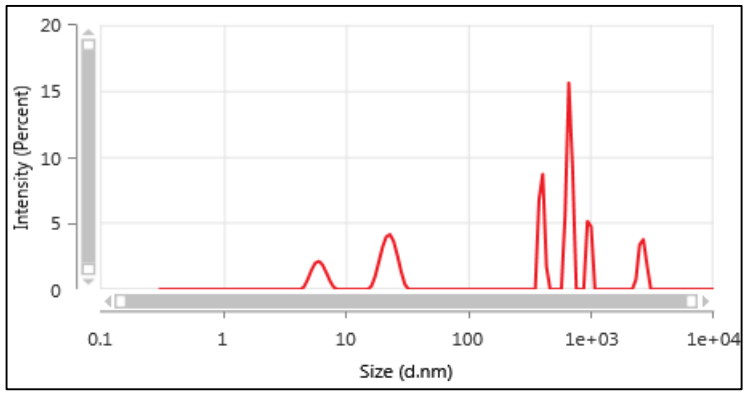
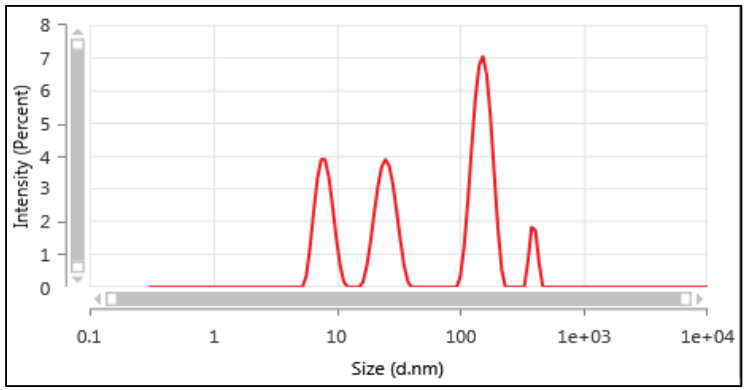
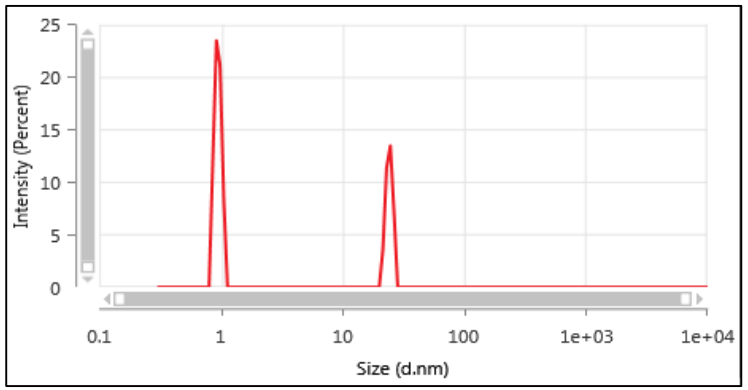
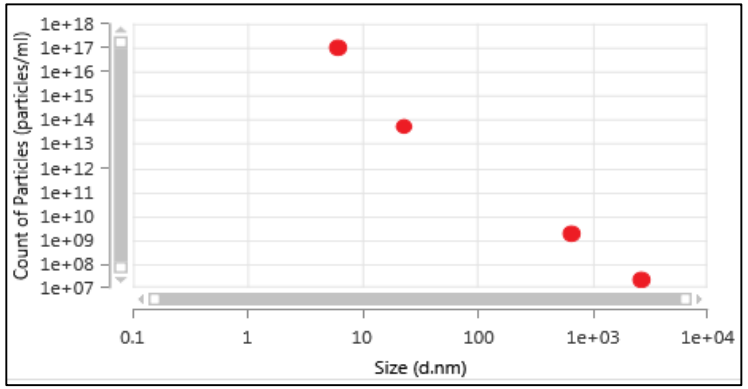
Total particle measure - MADLS

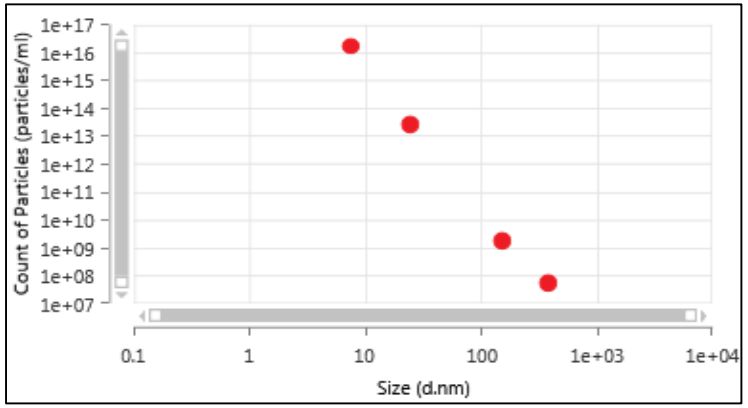
Lysis → Primary clarification → Secondary clarification → Affinity capture

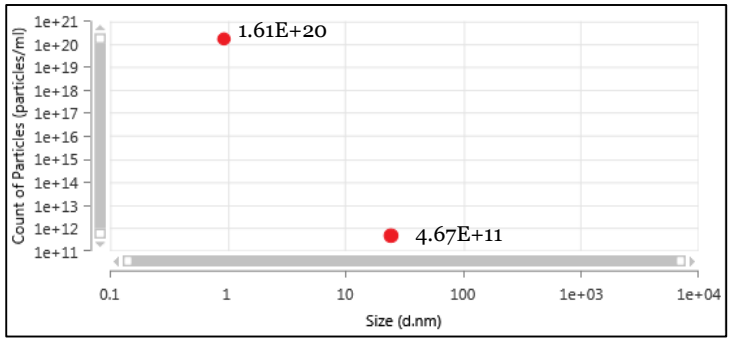












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CGT analytical capabilities and vision

CGT analytical capabilities

	1st Gen	2nd Gen
Physical titre	ELISA	MADLS
Packaged genomes	qRT-PCR	ddPCR
Packaging Ratio	ELISA/PCR	HPCE
Viral capsid proteins	Western blot	Automated WES
Aggregation	DLS	MADLS
Infectious titre	FACS	ddPCR
Functional titre	*In-vitro*	FACS/Impedance
Total protein	Coomassie	LC-MS
Sterility	Growth based	ddPCR
Purity		ddPCR / Seq

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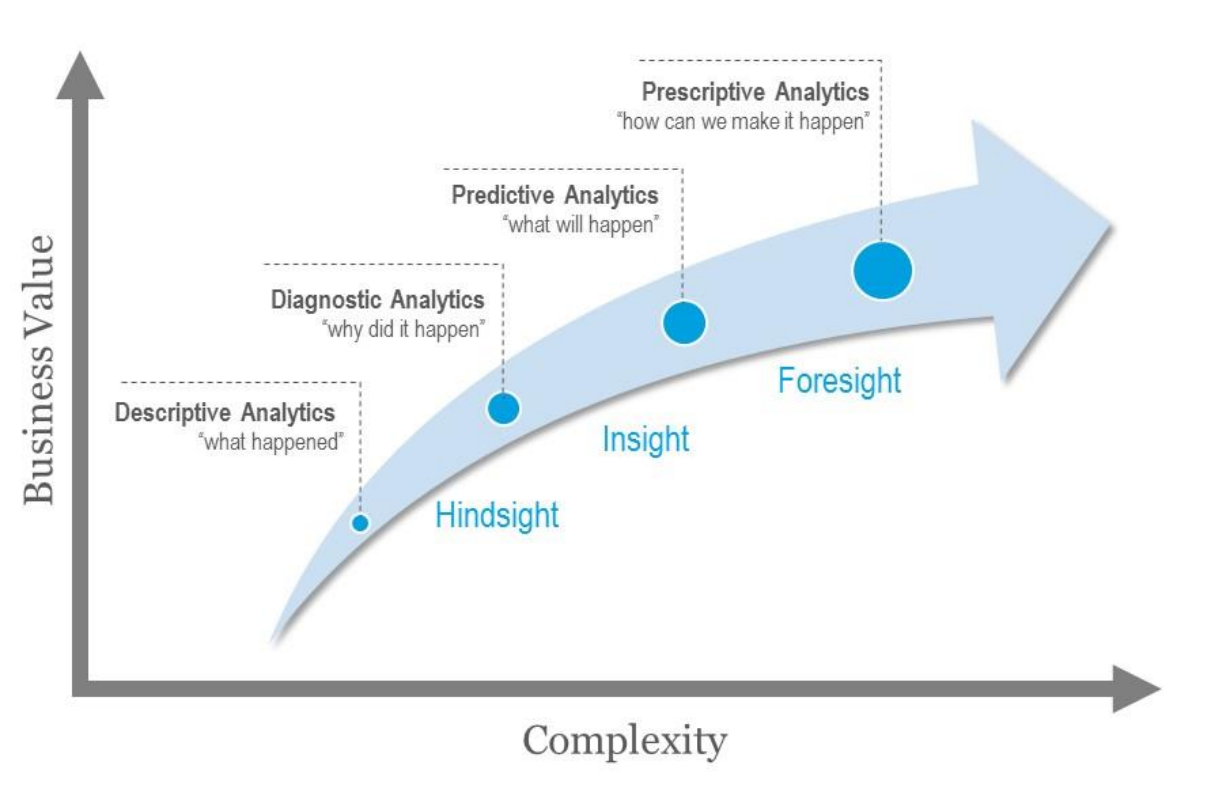
CGT vision

The data analytics continuum



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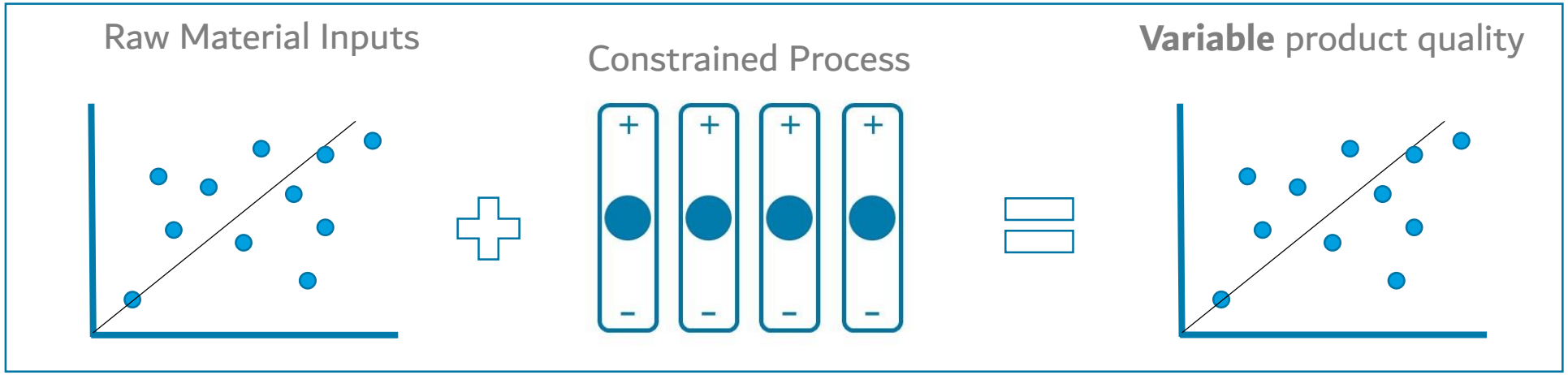
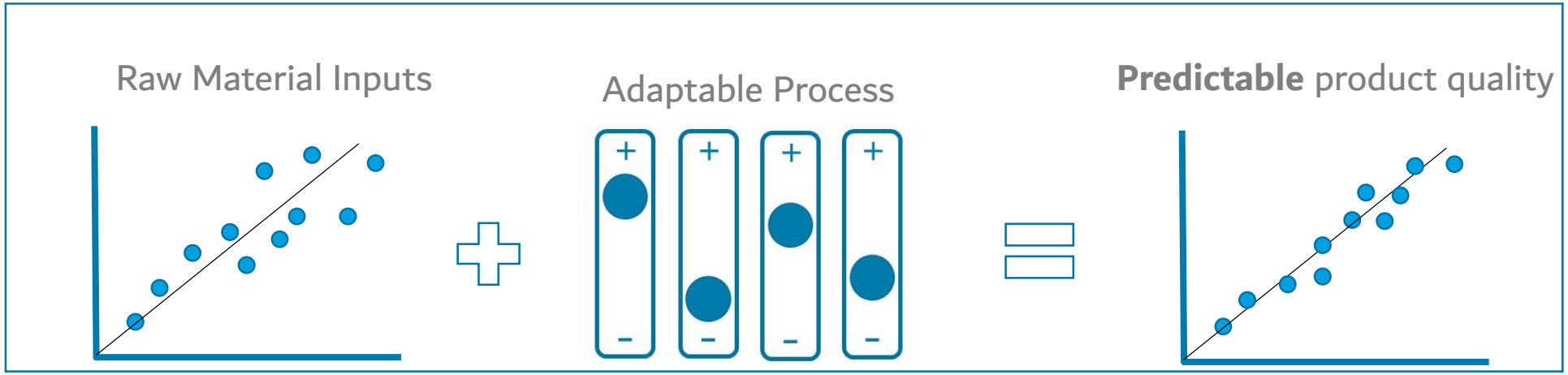
CGT vision

Now



Future



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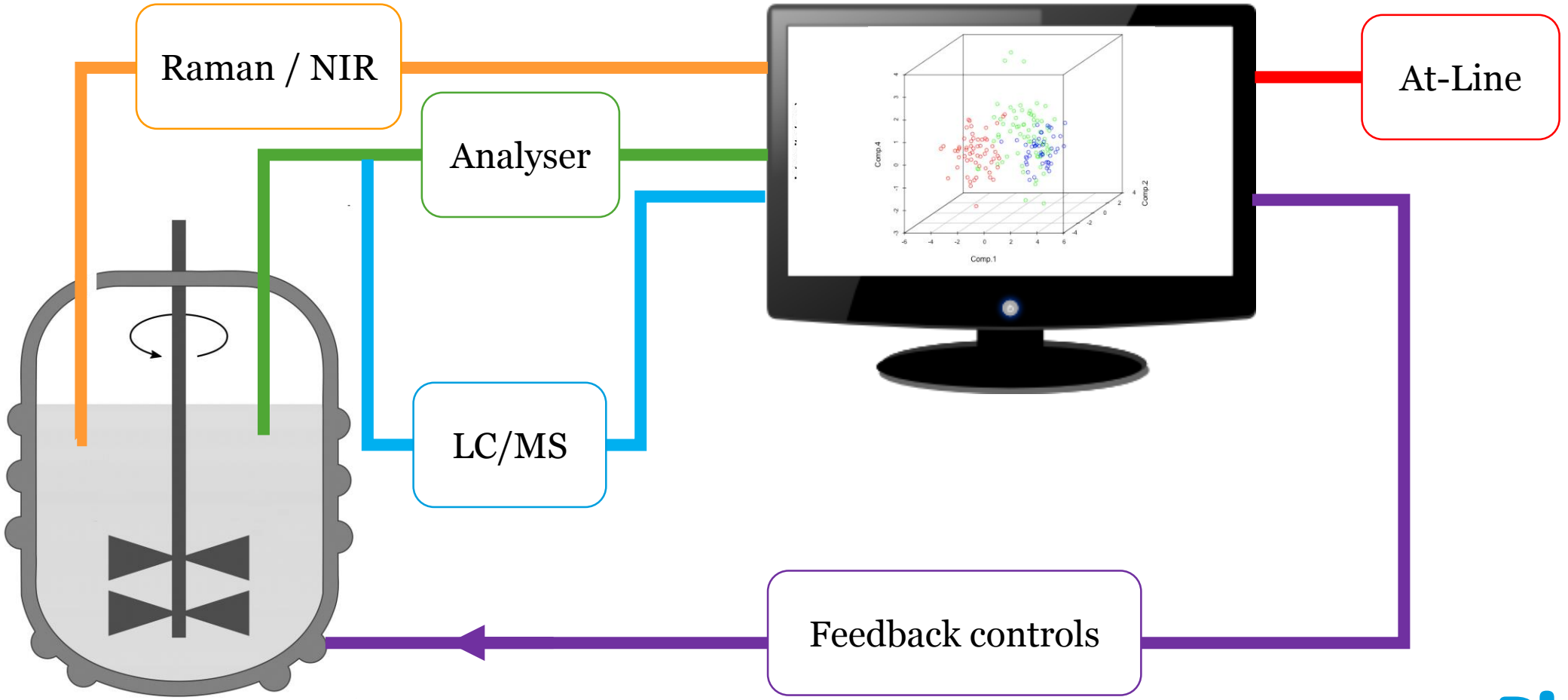
CGT Strategy



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