The next generation of AAV analytics

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Senior Analytical Scientist

Development of AAV therapeutics
KTN - Cambridge 30 Jan 2019
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.
Who we are

- **Cell Therapy catapult**
  - Centre of excellence in innovation

- **Cell and gene therapy Catapult - CGT**

- **Manufacturing centre**

**CGT Core projects**
- qPCR
- ddPCR
- DLS
- ELISA
- HPCE
- MADLS
- Western blot
- Automated WES
- FACS
- Infectious titre
- Sterility
- Impedance
- Physical titre
- Ovisio
- Transducing units
- LC-MS
- VCN
- Aggregation
- Purity
- Empty/full
- Raman
- Physiologic titre
- HCV
- Infectious titre
- Transducing units
- VCN
## Presentation workflow

<table>
<thead>
<tr>
<th>Quantity - Vector genome titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial qPCR testing kits</td>
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<tr>
<td>In-house ddPCR development</td>
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<tr>
<td>In-house qPCR development</td>
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<table>
<thead>
<tr>
<th>Quantity - Physical capsid titre</th>
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<tbody>
<tr>
<td>Testing of ELISA commercial kits</td>
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<tr>
<td>MADLS</td>
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<table>
<thead>
<tr>
<th>Purity</th>
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<tbody>
<tr>
<td>Full / Empty ratio</td>
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<table>
<thead>
<tr>
<th>Case study</th>
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<tbody>
<tr>
<td>Application of developed methods on an in-house production test run</td>
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</tbody>
</table>

| CGT analytical capabilities and vision |
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

![Graph showing quantity vector genome titre](image-url)
**Quantity – vector genome titre**

<table>
<thead>
<tr>
<th>Aim</th>
</tr>
</thead>
</table>

Develop a robust, accurate in-house method for measuring AAV2 vector genomes (and AAV2 derived serotypes)
## qPCR alternatives - ddPCR

<table>
<thead>
<tr>
<th>qPCR</th>
<th>ddPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image of qPCR" /></td>
<td><img src="image2.png" alt="Image of ddPCR" /></td>
</tr>
<tr>
<td>Amplification Curves</td>
<td><img src="image3.png" alt="Image of ddPCR" /></td>
</tr>
</tbody>
</table>

### qPCR
- **Amplification Curves**

### ddPCR
- Sample 1: No target
- Sample 2: Low concentration
- Sample 3: Medium concentration
- Sample 4: High concentration

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**CATAPULT**

Cell and Gene Therapy
## 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

- Expected copies
- Calculated copies
- R square: 0.9969
- Deviation from linearity: non significant
- LLoD: 16.16 copies
- LLoQ: 125 copies
- Intra/inter assay CV: < 20%
ddPCR and Commercial qPCR method comparability

ATCC® VR-1615

AAVpro extraction kit

Sample dilution

Sample prep with ITR primers & probe

Droplet generation

ddPCR

qPCR

Titre calculation & compare to initial known titre we started with & qPCR titre

Titre calculation & compare to initial known titre we started with & ddPCR titre

SELECTION AND COMPARABILITY

Calculate VR 1616 Titre

ddPCR results within 95% CI
## 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
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%

<table>
<thead>
<tr>
<th></th>
<th>% full particles</th>
<th>qPCR</th>
<th>ddPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>13.69%</td>
<td>13.69%</td>
<td>6.72%</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>13.69%</td>
<td>13.69%</td>
<td>6.72%</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>13.69%</td>
<td>13.69%</td>
<td>6.72%</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>13.69%</td>
<td>13.69%</td>
<td>6.72%</td>
</tr>
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</table>

**Limitations**

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

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**Quantity/purity – total particle measure/ Empty to full ratio**
Dynamic Light Scattering technology

- **Backscatter (173°)**
  - (High conc.)
- **Particles moving due to Brownian motion**
- **Zeta potential**
- **90° for size and MW, A2**
- **Laser**
  - 532nm, 10mW
- **Attenuator**
- **Microliter**

**DLS Range**
- Water
- Glucose
- Antibody
- Virus
- Bacteria
- Cancer Cell
- Period
- Tennis Ball

**Graphs:**
- Intensity (percent) vs. Size (nm)
- Count of Particles (particles/ml) vs. Size (nm)
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
Case study
Overview of downstream developmental scope

- **Cell disruption**
  - Viral vector release
devolution 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

- **Capture and polishing**

- **Formulation concentration**

- **Sterile filtration**
  - Fill and Finish

- **Source nucleases**
  - Optmise treatment.

- **Cell lysis methods.**
  - Filter options

- **Cell lysis methods.**
Cell Lysis Experiment – Study Design & Workflow

Harvest

Physical lysis

DNase treatment

Physical lysate

Harvest

Centrifugation

Extraction buffer

Freeze/thaw cycles

DNase treatment

Lysate

Chemical lysis

Chemical lysate
AAV titre and purity check

<table>
<thead>
<tr>
<th>Physical lysis</th>
<th>Chemical lysis</th>
<th>Freeze / thaw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Particle number produced</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAV titre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 10^{10} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 10^{11} )</td>
<td></td>
<td></td>
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<tr>
<td>( 10^{12} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 10^{13} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sample purity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vg (ddPCR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vg (qPCR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (ELISA)</td>
<td></td>
<td></td>
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<td><strong>Full to empty ratio</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Full / Empty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
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</tr>
<tr>
<td>150</td>
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</tbody>
</table>

Particle number produced

Sample purity
Total particle measure - MADLS

1. Lysis
2. Primary clarification
3. Secondary clarification
4. Affinity capture

Graphs showing particle size distribution and count for each step in the process.
CGT analytical capabilities and vision
**CGT analytical capabilities**

<table>
<thead>
<tr>
<th></th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Gen</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Gen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical titre</td>
<td>ELISA</td>
<td>MADLS</td>
</tr>
<tr>
<td>Packaged genomes</td>
<td>qRT-PCR</td>
<td>ddPCR</td>
</tr>
<tr>
<td>Packaging Ratio</td>
<td>ELISA/PCR</td>
<td>HPCE</td>
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<td>Viral capsid proteins</td>
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<td>MADLS</td>
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<tr>
<td>Infectious titre</td>
<td>FACS</td>
<td>ddPCR</td>
</tr>
<tr>
<td>Functional titre</td>
<td>In-vitro</td>
<td>FACS/Impedance</td>
</tr>
<tr>
<td>Total protein</td>
<td>Coomassie</td>
<td>LC-MS</td>
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<tr>
<td>Sterility</td>
<td>Growth based</td>
<td>ddPCR</td>
</tr>
<tr>
<td>Purity</td>
<td>ddPCR / Seq</td>
<td></td>
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</table>

- **Plasmids**
- **Cell debris**
- **Free genomes**
CGT vision

The data analytics continuum

- Descriptive Analytics
  - "What happened"

- Diagnostic Analytics
  - "Why did it happen"

- Predictive Analytics
  - "What will happen"

- Prescriptive Analytics
  - "How can we make it happen"

- Foresight
- Insight
- Hindsight
**CGT vision**

**Now**
- **Raw Material Inputs**
- **Constrained Process**
- **Variable** product quality

**Future**
- **Raw Material Inputs**
- **Adaptable Process**
- **Predictable** product quality
CGT Strategy

Raman / NIR

Analyser

LC/MS

Feedback controls

At-Line
Acknowledgements

Julie Kerby
Damian Marshall
Mike Delahaye
Gregory Berger
Nicole Nicolas
Anusha Seneviratne
Nishanthi Weeratunge
Florian Leseigneur
Quentin Bazot
Elena Sokolskaja