



# Development of a GMP-compliant Mass Photometry Platform Method for AAV Capsid Population Analysis

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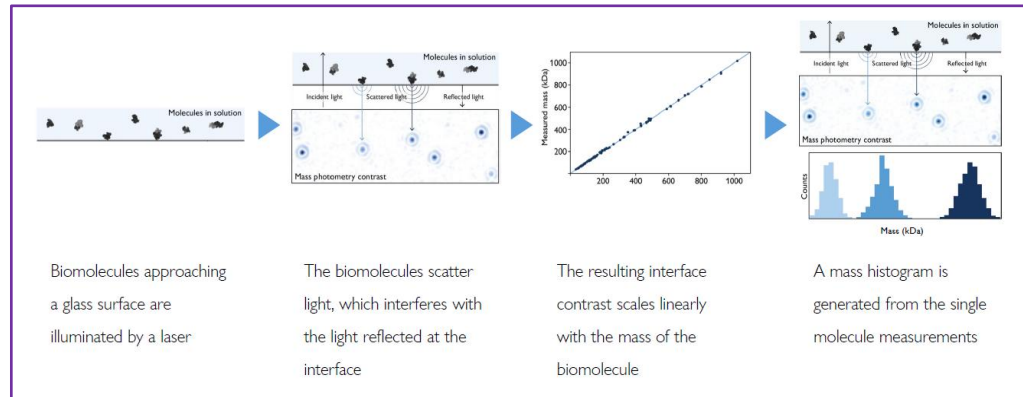
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# Mass Photometry

## An Orthogonal QC-friendly tool for AAV Capsid population Analysis

### Principle of MP



### MP Histogram

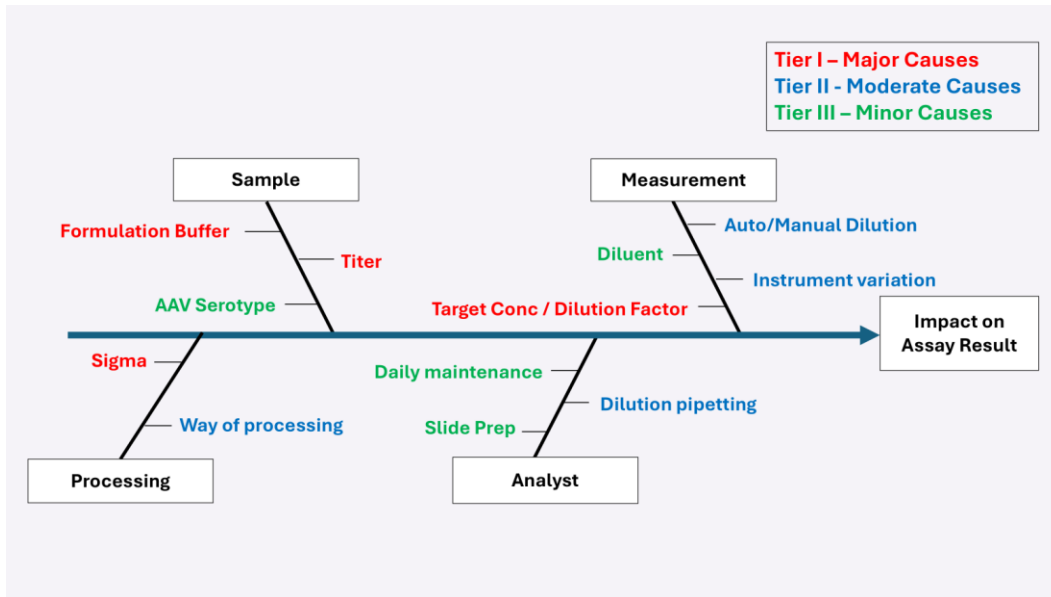


### Alignment with AUC Results

| Program A   | MP | AUC | Program B   | MP | AUC | Program C   | MP | AUC |
|-------------|----|-----|-------------|----|-----|-------------|----|-----|
| Batch 1, E% | 15 | 10  | Batch 1, F% | 79 | 80  | Batch 1, F% | 75 | 75  |
| Batch 1, P% | 32 | 35  | Batch 2, F% | 82 | 83  | Batch 2, F% | 71 | 72  |
| Batch 1, F% | 53 | 55  | Batch 3, F% | 78 | 79  | Batch 3, F% | 71 | 68  |
|             |    |     | Batch 4, F% | 80 | 81  | Batch 4, F% | 71 | 71  |
|             |    |     |             |    |     | Batch 5, F% | 70 | 71  |

# Method Development

## Ishikawa Diagram for Method Development



## Selected Development and Method Performance Studies

1. Proof of Concept
2. Processing Method assessment
3. Algorithm assessment
4. Linearity of Final Conc. vs Binding Count
5. Intermediate Precision - Analysts (n=3)
6. Intermediate Precision - Instruments (n=2)

# Pre-Qualifications

## Outcome of Pre-Qualification Program A

| Attribute              | Parameter                                                                                | Analyst A Result | Analyst B Result |
|------------------------|------------------------------------------------------------------------------------------|------------------|------------------|
| Specificity            | Binding Count within AAV range in formulation buffer                                     | 4                | 4                |
| Linearity              | Linear calibration curve's R2 of E/P/F                                                   | 0.97, 0.99, 1.00 | 0.98, 0.96, 0.99 |
| Repeatability          | RSD% of %Full of all 3 measurements at 3 levels (L,M,H)                                  | 2%, 5%, 4%       | 5%, 7%, 1%       |
| Intermediate Precision | RSD% of %Full of all 6 measurements of each sample (from 2 analysts) at 3 levels (L,M,H) | 5%, 6%, 4%       |                  |
| Accuracy               | Inferred once precision, linearity and specificity have been established.                | Pass             | Pass             |

## Outcome of Pre-Qualification Program B

| Attribute              | Parameter                                                                                | Result       |
|------------------------|------------------------------------------------------------------------------------------|--------------|
| Specificity            | Binding Count within AAV range in formulation buffer                                     | 2            |
| Linearity              | Linear calibration curve's R2 of Full%                                                   | 0.99         |
| Repeatability          | RSD% of %Full of all 3 measurements at 3 levels (L,M,H)                                  | 4%           |
| Intermediate Precision | RSD% of %Full of all 6 measurements of each sample (from 2 analysts) at 3 levels (L,M,H) | 4%           |
| Accuracy               | Inferred once precision, linearity and specificity have been established.                | Pass         |
| LOQ                    | LOQ is the lowest level where 3 measurements of %Full demonstrate RSD $\leq$ 15%.        | 5.9%         |
| Range                  | Range spans from LOQ to highest %Full where precision and linearity are acceptable.      | 5.9% - 87.4% |

# Summary

1. A robust platform method was developed for % Full AAVs.
2. Final method demonstrated robust method performance.
3. Effective communication and sharing of knowledge with vendor was key to the successful method development.