Considerations for AAV analytical comparability studies for products with low batch numbers

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Poster 0220

Abstract

Manufacturing changes are often implemented during the development of AAV gene therapies. These changes may be significant, for example changing the manufacturing platform to generate a scalable manufacturing process, or much smaller, such as transferring an existing process from one manufacturing site to another.

These manufacturing changes must be accompanied by comparability studies demonstrating that the post-change product has an equivalent safety and efficacy profile to the pre-change product. If analytical comparability can be demonstrated based on a good understanding of product critical quality attributes (CQAs) and using methods that can provide high assurance of safety and efficacy, then repetition of preclinical toxicity or human dose-finding and efficacy studies may not be needed.

Several guidance and draft guidance documents are available from various agencies to guide the comparability process. Ideally, a large number of pre- and post-change batches should be compared to provide statistical assurance that the change(s) introduced do not affect product CQAs. However, since many AAV gene therapies are often produced for rare diseases with relatively low numbers of patients and since batch manufacturing costs are high, a limited number of batches is normally available.

Here we present examples of comparability plans which are compatible with current guidance, and which are tailored to AAV gene therapies. This includes considerations for CQA risk assessments when changes are made to different parts of the process, e.g. upstream, downstream or formulation, as well as strategies for generating data that provides sufficient statistical assurance of comparability using only a small number of pre- and post-change batches.

Process Flow

1. **Process Change**
   - Improvement
   - Scale-up
2. **Risk Assessment**
   - Identify CQAs
   - Risk assess each CQA
3. **Comparability Test Plan**
   - Number of batches
   - Side-by-side testing
4. **Acceptance Criteria**
   - Statistical methods
   - Nr. of test occasions
5. **Comparability Assessment**
   - Perform testing
   - Statistical analysis
6. **Comparability Result**
   - Assess comparability
   - Assess differences

If analytical comparability can be demonstrated based on a sound plan and suitable analytical methods, clinical and non-clinical studies can be avoided

CQA Risk Assessment

Score	Probability (P)	Severity (S)	Detectability risk (D)
Low	Unlikely (< 2%)	Low potential to affect safety or efficacy	Methods reliably detect changes in the attribute with high precision and accuracy
Med	Moderately likely (2-20%)	Safety or efficacy may be impacted, but (i.e. non-serious adverse events, small changes in efficacy)	Methods are available but may suffer from limited sensitivity precision or accuracy
High	Highly likely (> 20%)	Safety or efficacy may be significantly altered (i.e. serious adverse events, significant changes in efficacy)	Methods are not available or do not have suitable sensitivity precision or accuracy

Tier 3 → Specification

Tier 2 → Quality Range

Tier 1 → Significance Testing

Each attribute is assessed for the probability, severity and risk of not detecting a change. Based on the scores, the attributes are assigned to a tier (i.e. 2x low + 1 x med ( ) = Tier 3) and each tier is assigned an appropriate assessment of comparability

Examples of CQA risk assessments

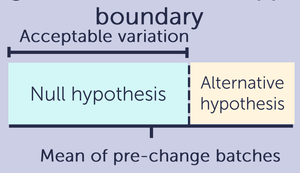
Example 1: Upstream Changes, e.g. Improved plasmid design

Attribute	P	S	DR	Tier
Packaged DNA Impurities	H	H	L	1
Post-translational modifications	M	M	L	2
Potency	H	H	M	1
Process Residuals	L	M	L	3
Aggregation	L	M	L	3
% full capsids	H	H	L	1
Device compatibility	L	H	L	3
Stability	L	H	L	3

Packaged DNA impurities: a Tier 1 attribute

- **Significance testing with an upper boundary** demonstrates that the mean levels of packaged impurities from the post-change process are not higher than those of the pre-change process within an acceptable margin, e.g. 10%
- Even a reduction in impurities <u>may</u> result in products being considered not comparable[1], so **a risk assessment** will be required to justify a one-sided test

Significance test with upper boundary



Always consult with a statistician when developing a comparability plan

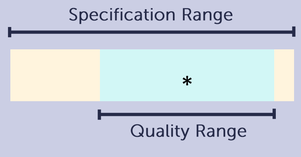
Example 2: Downstream Changes, e.g. Addition of a Virus Filtration Step

Attribute	P	S	DR	Tier
Packaged DNA Impurities	L	M	L	3
Post-translational modifications	L	M	L	3
Potency	L	H	M	2
Process Residuals	M	M	L	2
Aggregation	M	M	L	2
% full capsids	L	H	L	3
Device compatibility	L	H	L	3
Stability	L	H	L	3

Aggregation: a Tier 2 attribute

- A suitable **quality range** will be defined, which all post-change batches must fall within in order to be considered comparable with respect to this attribute
- As an example, the range may be defined by the mean of the pre-change batches ± 1.5 standard deviations
- The range should typically be **tighter** than the **specification limits**

Quality Range



* Mean of pre-change batches

Size of the quality range depends on severity of the CQA and on precision of the method

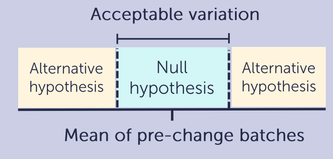
Example 3: Formulation Changes, e.g. Addition of surfactant

Attribute	P	S	DR	Tier
Packaged DNA Impurities	L	M	L	3
Post-translational modifications	L	M	L	3
Potency	M	H	M	1
Process Residuals	L	M	L	3
Aggregation	M	M	L	2
% full capsids	L	H	L	3
Device compatibility	H	H	L	1
Stability	H	H	L	1

Potency: a Tier 1 attribute

- **Equivalence testing using the two one-sided t-test (TOST)** procedure demonstrates that the mean potency from the post-change batches is comparable within a defined margin (e.g. ±30%) to the mean potency of the pre-change batches
- A quantitative potency assay with **good precision** is required to be able to determine comparability using this approach.
- See also Poster **0068** for potency assay development considerations

Two one-sided t-test (TOST)

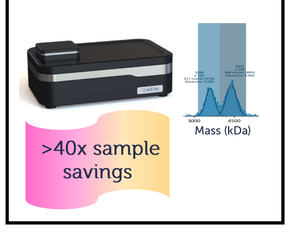


Measuring each batch multiple times can improve precision, particularly useful for small numbers of batches*

* When measuring a batch multiple times, the average value for the batch is used in statistical testing, not the individual measurements

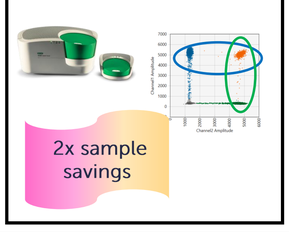
Use of low volume methods

AUC ➥ Mass Photometry



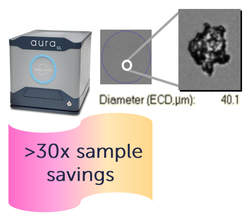
>40x sample savings

Singleplex➥Duplex ddPCR



2x sample savings

Light Obscuration ➥ BMI



>30x sample savings

ELISA ➥ Gyros

4x sample savings

Pacbio ➥ Nanopore

10x sample savings

Low volume methods can benefit comparability

- Side-by-side testing is recommended[1, 2]
- Performing an assay multiple times per sample can be used to reduce measurement uncertainty[1]
- These recommendations can only be implemented if sufficient sample is available
- Many of these assays also provide cost reductions and improvements in throughput
- See also Poster 0057 for validation of ddPCR and Gyros and Poster 0197 for duplex ddPCR

References

1. FDA: Manufacturing Changes and Comparability for Human and Cellular Gene Therapy Products, Draft Guidance for Industry July 2023
2. FDA: Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products April 1996
3. ICH Q5E: Comparability of Biotechnological/biological Products Subject to Changes in their Manufacturing Process

Summary

This poster has provided examples of comparability changes to AAV manufacturing processes. Changes to upstream, downstream and formulation were considered and examples of select CQA classifications were provided for which different statistical approaches may be taken, commensurate to the risk posed by the CQA.

We have also discussed ways to improve data quality when small numbers of batches are available, including the use of low volume methods which can enable side-by-side testing and testing of the same sample multiple times.

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