GILEAD
Creating Possible

A QbD Approach for Analytical Comparability of an Antibody Drug Conjugate

CASSS CMC Strategy Forum 2025

Analytical Comparability in Biologics

- Analytical comparability is a systematic, science- and risk-based assessment to ensure that **manufacturing changes** (e.g., process scale-up, site transfer, raw material changes) do not adversely impact the product's quality, safety, or efficacy.

- The goal of comparability is to demonstrate that the pre- and post- change products are "**highly similar**" in physicochemical and biological properties, with no clinically meaningful differences.

- Regulatory expectations emphasize a science- and risk-based approach, aligned with **ICH Q5E** and regional guidance. It's the foundation for approving manufacturing changes without new clinical trials.

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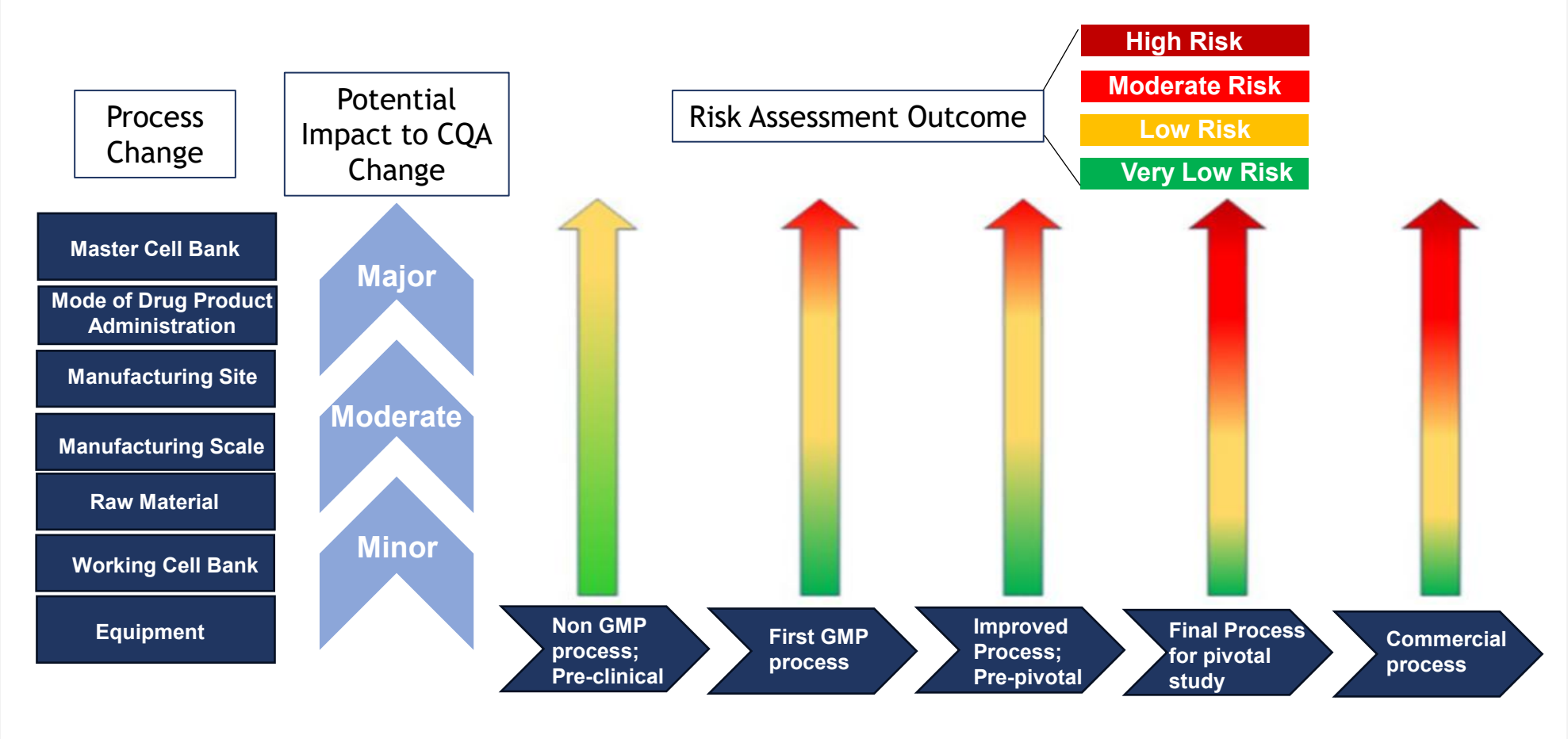
When is Comparability Required

Comparability is necessary for **process changes** made throughout the lifecycle of biologics development.



The bar for evidence rises across the lifecycle. Always anchor decisions in **Patient Impacts**.

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Figure courtesy: Journal of Pharmaceutical Sciences 113 (2024) 1415–1425

QbD Enhanced Analytical Comparability Framework

- ❑ Tier CQA to Clinical Criticality
- ❑ Process Risk Assessment to Define Testing Strategy
- ❑ Patient-Centered Comparability Criteria to Ensure Patient Safety and Efficacy
- ❑ ATP-Driven Analytical Method Development

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CQAs are the Foundation for Performing Comparability Assessment

Critical Quality Attributes (CQAs) — ICH Q8R2

*Chemical, biological, or microbiological **property or characteristic** that should be within an **appropriate limit, range, or distribution** to ensure the desired **product quality**.*

Criticality =
Impact x Uncertainty

- Biological Activity
- PK/PD
- Immunogenicity
- Safety

- Clinical Data
- Literature
- Prior Knowledge

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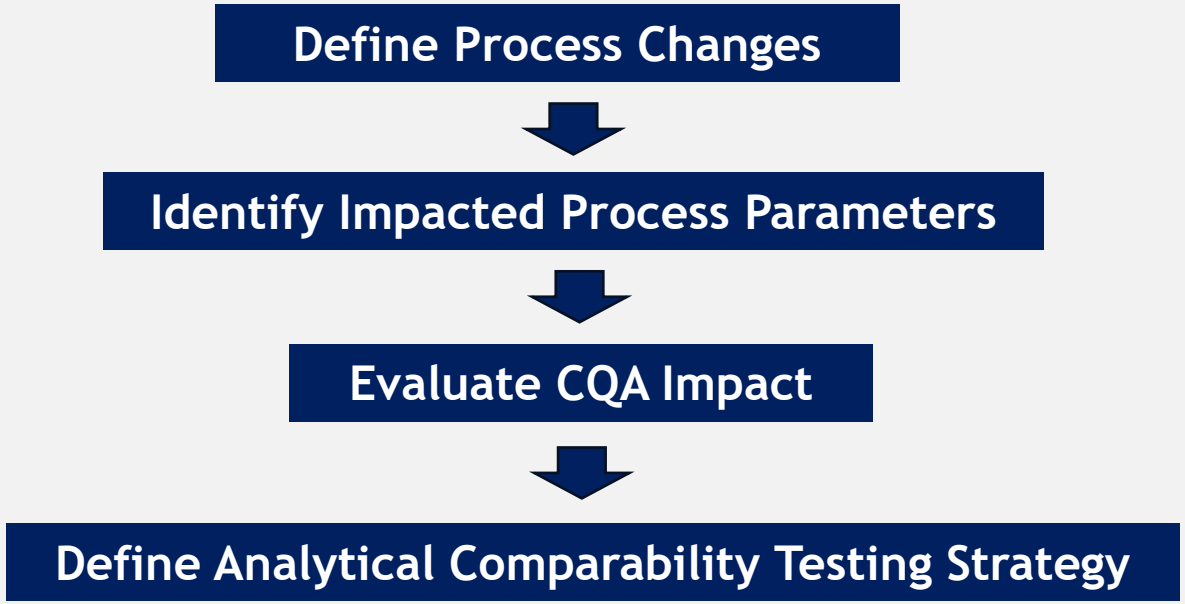
Process-Based Risk Assessment

- Linking a process change to CQA is a **core step** in risk-based analytical comparability.
- The aim is to understand **how and why** a change may impact product quality and to justify the analytical testing strategy



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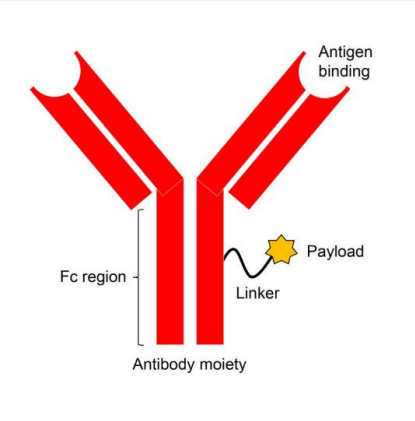
Linking Process Changes to CQAs in an ADC

Process Change: Adjustment of drug-to-antibody ratio (DAR) target by modifying the molar ratio of drug-linker to antibody and reaction condition

ADC Structure



Process Step	Affected Process Parameters	Justification for CQA Impact	Potentially Impacted CQAs				
			DAR/Drug-load Distribution	Off-target Conjugation	Free Payload	Aggregation	Potency
Conjugation	Increased molar Ratio	May cause higher DAR species	X	X			X
	Increased reaction time	Risk of over-conjugation, Aggregation	X	X		X	X
	Higher temp	May cause aggregation or payload loss	X		X	X	X

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Figure courtesy: Drug Discovery and Biotech

CQAs-Focused Comparability Testing Strategy

Process Risk Assessment: Evaluation of the potential impact of process / site changes to CQAs

Release Assessment: Post change lots are compared to historical clinical lots both quantitatively and qualitatively

Extended Characterization: CQAs that may be impacted are evaluated both qualitatively and quantitatively

Side by Side Stress Stability: Evaluation if similar degradation is observed for Pre-Change and Post-Change Lots at Relevant Stress Conditions

Pre- and Post-Change lots are highly similar and any differences do not have a negative impact on efficacy and patient safety

Critical Quality Attributes

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Comparability-Release Testing

Purpose:

- ❑ To confirm that post-change bathes meet specification and remain within historical or clinically justified ranges for CQAs, ensure consistent product quality, efficacy and safety

Key Notes:

- ❑ ≥ 3 post-change batches to confirm CQA consistency
- ❑ Acceptance ranges should be derived form clinical/commercial batch data. Support criteria with appropriate statistical methods. Set more stringent criteria for critical attributes that influence efficacy and safety
- ❑ Assess profiles for purity method: confirm no new peaks and same rank order

Impacted CQAs	Clinical Relevance	Comparability Criteria	Criteria Setting Rationale
Aggregate (SEC-HPLC)	Safety Risk	No new peaks and same rank order for pre- and post- change lots ≤ SPEC or Historical + 3SD, whichever is tighter	Attribute has tight specification, and more stringent historical trend is known
Cell-based bioassay	Efficacy	Historical mean ± 3SD	Attribute shows low varability and has sufficient historical data
Free payload	Off-target toxicity	≤ Clinical Max	Attribute has direct clinical relevance, especially related to toxicity, fixed upper limit ensures patient safety
DAR (average)	Safety and Efficacy	Clinical Min-Max	Attribute is critical for safety and efficacy

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Comparability-Extended Characterization

Purpose:

- ❑ Extended characterization focused on attributes not routinely monitored but still critical for understanding comparability

Key Notes:

- ❑ At least 3 pre-change vs 3 post-change batches recommended
- ❑ Focus on CQA-relevant attributes using orthogonal and high-resolution methods when appropriate
- ❑ Establish comparability criteria with rationale tied to patient impact

CQAs	Method	Clinical Relevance	Comparability Criteria	Criteria Setting Rationale
DAR Distribution	Native MS, HIC, etc.	Impact efficacy, safety or PK	Match major DAR species % and %CV<10% across lots	Ensures consistent drug delivery, potency, and PK behavior to maintain therapeutic effect
Off-Target Conjugation	rPeptide Mapping	Unintended toxicity or altered clearance	Below method LOQ	Prevents unintended toxicity due to mis-conjugation
Conjugation Process Impurities	HPLC-UV, GC etc.	Toxicity from residual solvents or linkers	Meet predefined safety limit based on tox studies	Prevents unintended toxicity due to residuals

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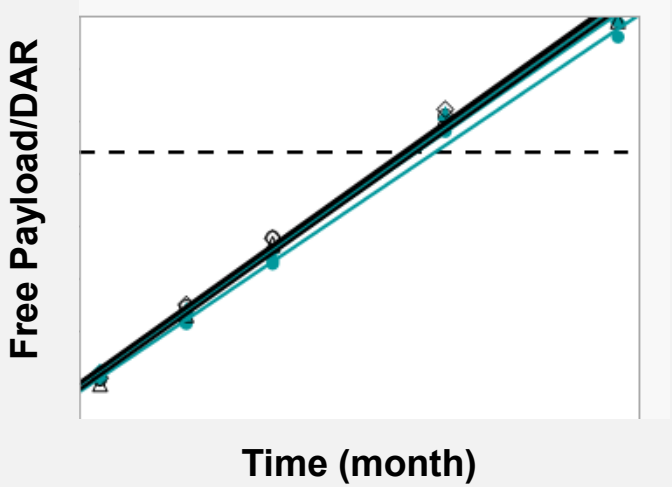
Side by Side Stress Stability

Purpose:

- ❑ To evaluate degradation behavior of pre- and post- change materials under controlled stress condition, focusing on:
  - ♦ Degradation pathways
  - ♦ Degradation rate (i.e., slope comparisons)
  - ♦ Sensitive CQAs and stability-indicating attributes

Key Notes:

- ❑ Controlled comparisons: Testing conduced under identical conditions and same timepoints for all bathes to minimize analytical variability
- ❑ Stress conditions: Use clinically relevant stress (i.e., thermal stress) to simulate accelerated degradation
- ❑ Stress duration: Long enough to reveal meaning degradation trends, but not too long in cause excessive degradation or noise
- ❑ Statistical rigor: Apply poolability analysis etc, focusing only on sensitive CQAs to reduce noise and avoid over-interpretation



- ♦ 3 Pre-change Batches vs. 3 Post-change Batches
- ♦ Thermal stress, 5 time points (i.e., 0, 1, 2, 3, 6 weeks)
- ♦ Linear regression on degradation slope, $p \geq 0.25$ (no statistically significant difference)

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CQA/ATP Driven Analytical Method Development

CQAs	ATP Examples	Method Options
Average DAR	Accurately and precisely quantify the average DAR for each batch Performance Criteria: - Accuracy: ±10% - Precision: %RSD ≤ 10% across replicate injections - Specificity: No interference from unconjugated antibody or free drug - Linearity: r^2 ≥ 0.99 across expected DAR range	UV/Vis, HIC, etc.
DAR Distribution	Resolve and quantify species with different DAR values, be able to detect any new DAR species Performance Criteria: - Resolution: Baseline separation of adjacent DAR peaks - Quantitation: Each DAR species quantifiable ≥ LOQ - Detection: Each species detectable ≥ LOD - Repeatability: %RSD ≤ 10% for each major DAR species	native MS, HIC, etc.

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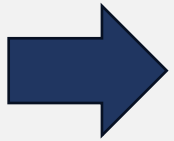
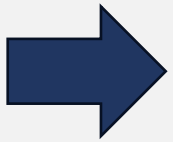
Summary of QbD Enhanced Analytical Comparability Approach

- The QbD approach starts with a comprehensive risk assessment to identify **CQAs** potentially impacted by process changes.



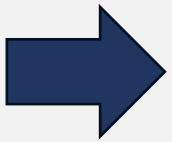
- This ensures that the comparability plan is scientifically justified and focused on product attributes most relevant to safety and efficacy.

- An **integrated testing strategy with patient-centered acceptance criteria** are applied to compare pre- and post- change products, focused on CQAs related to clinical performance, structure-function integrity, and degradation behavior.



- This provides a flexible yet rigorous framework to support regulatory confidence and ensures effective lifecycle management for complex modalities like ADCs.

- Analytical methods are selected and developed based on the **ATP (linked to CQAs)** to ensure they are fit for purpose.



- This supports robust detection of meaningful differences and ensures method suitability across the product lifecycle.

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