Rethinking qPCR Validation in Regulated Bioanalysis?
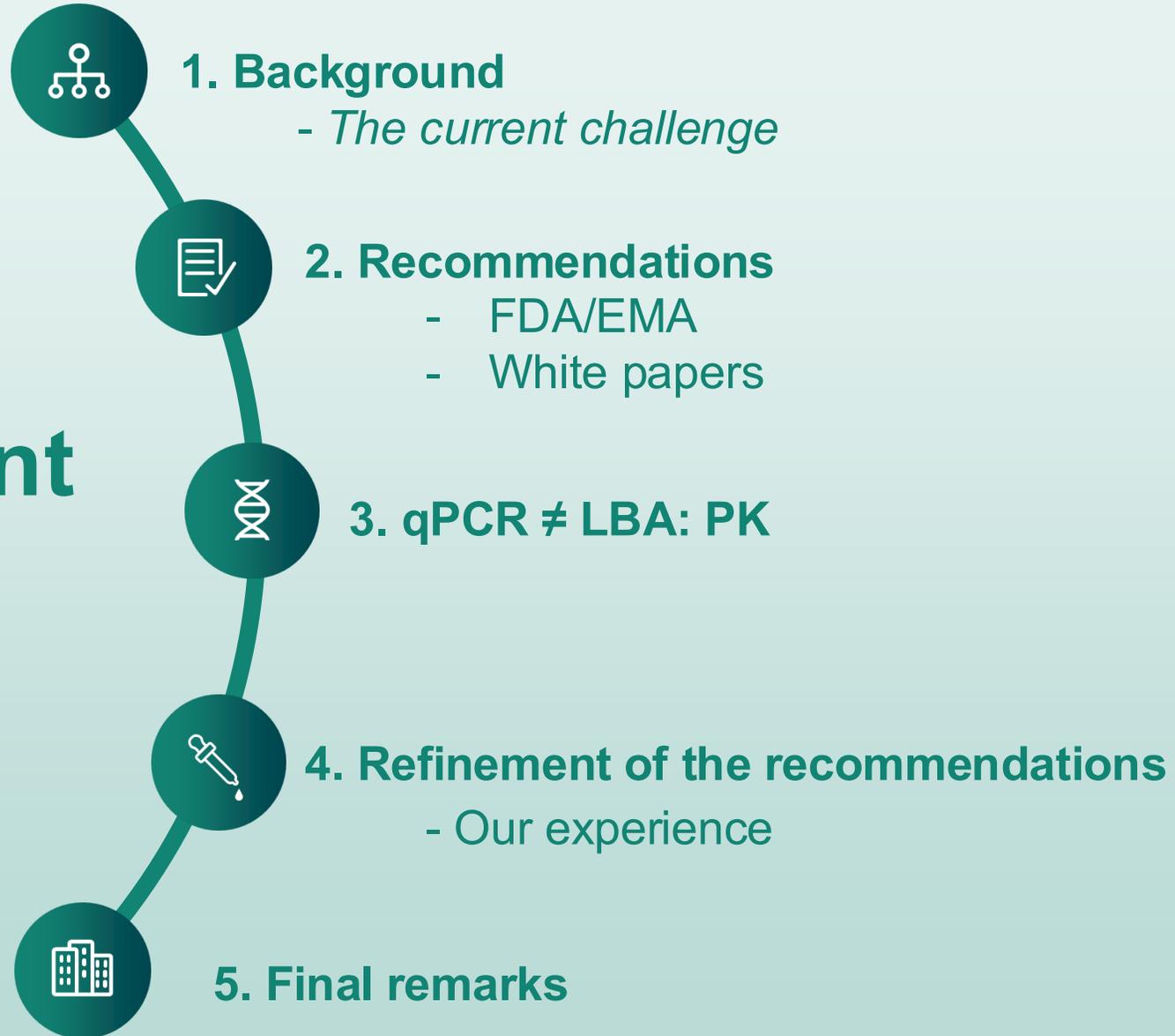
Practical Guidance Beyond LBA Principles

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18th EBF Open Symposium

Content

Background

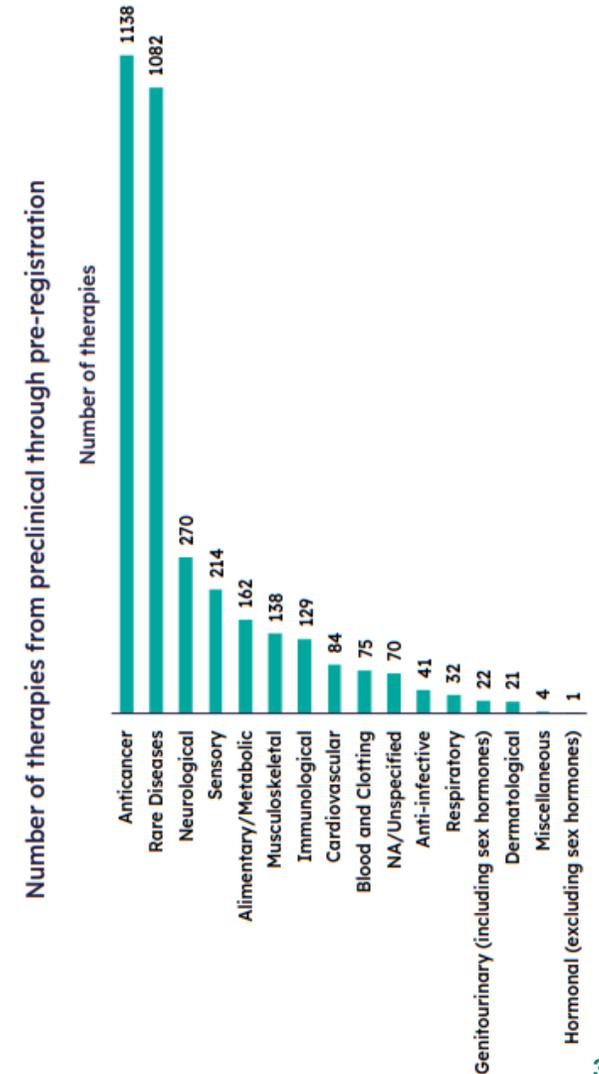**Gene and cell therapy programs have continued to increase**

Pipeline overview: +4400 therapies in development

Oncology and rare diseases are top areas of development

Molecular biology-based technologies: important platform for bioanalysis supporting drug development

American Society of Gene + Cell therapy, Q2 2025 Quarterly Data Report

Number of therapies from preclinical through pre-registration
Number of therapies

Category	Number
Anticancer	1138
Rare Diseases	1082
Neurological	270
Sensory	214
Alimentary/Metabolic	162
Musculoskeletal	138
Immunological	129
Cardiovascular	84
Blood and Clotting	75
NA/Unspecified	70
Anti-infective	41
Respiratory	32
Genitourinary (including sex hormones)	22
Dermatological	21
Miscellaneous	4
Hormonal (excluding sex hormones)	1

Background

The current challenge

01

Bioanalytical laboratories/CROs are undertaking validation of qPCR assays
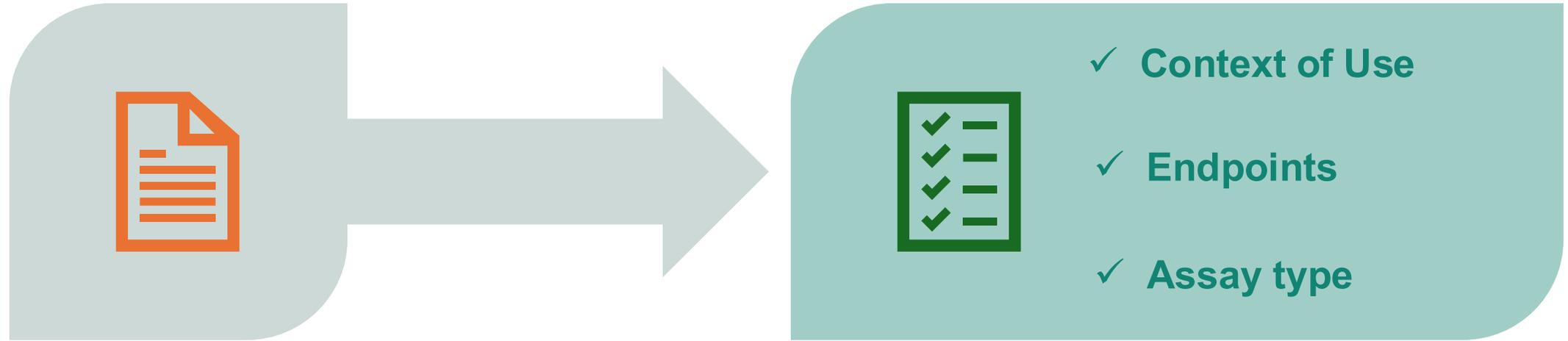
02

No harmonized regulatory guidance for qPCR validation / bioanalysis

03

Fact: Almost 90% of the qPCR/dPCR assays are performed in regulated bioanalysis environment

Rethinking?

The current challenge

From:

- White Papers

To:

✓ **Context of Use**

✓ **Endpoints**

✓ **Assay type**

- Guidelines…?
- ***Refinement of White Papers***
(Tailored to qPCR not LBA concepts)

The recommendations

01 Guideline

- Guidance for Industry. ICH M10. *Issued in 2022*

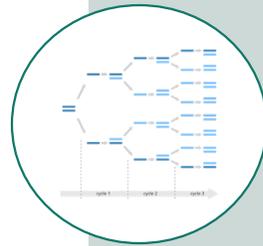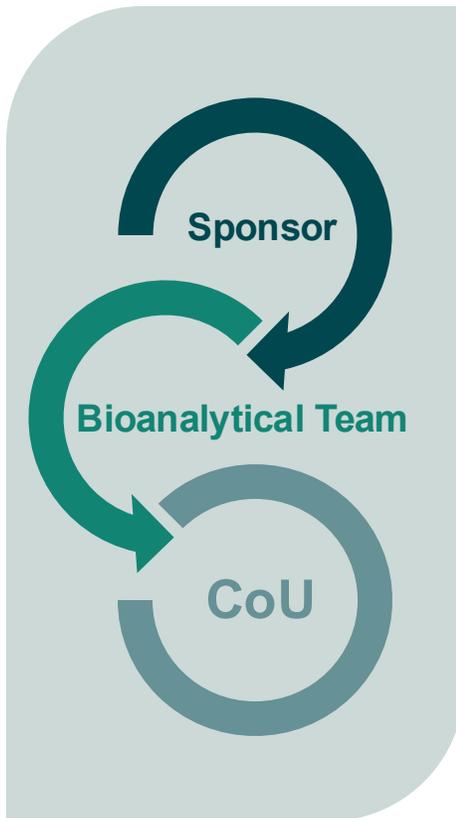- Bioanalytical Method Validation for Biomarkers - Guidance for Industry. *Issued in January 2025…*?

02 White Papers

- Often extrapolate ligand-binding assay (LBA) validation principles
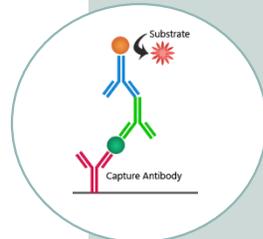
- May not fully reflect qPCR's unique analytical nature

Why qPCR ≠ LBA: PK Assay

For fit-for-purpose

Sponsor
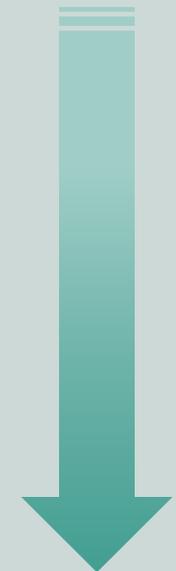
Bioanalytical Team

CoU

qPCR

- Extraction of nucleic acids
- Exponential amplification
- Analyte: Amplification based
- Quantitation relative or absolute

LBA: PK

- Serum/plasma directly
- Signal linear or sigmoidal
- Analyte: Direct detection
- Always absolute (concentration)

Qualification

Validation

Refinement of Recommendations

Verification Level:

| Qualification | Partial Validation | Full Validation |

Endpoints:

| Exploratory | Secondary | Primary |

Study type:

Qualitative / Semiquantitative

- ✓ Gene expression
- ✓ SNPs

Quantitative

- ✓ Biodistribution
- ✓ Target gene expression
- ✓ Viral shedding

- ✓ Cellular Kinetics

Parameter:

Tailored depending on CoU

Refinement of Recommendations

Qualification		Partial Validation		Full Validation
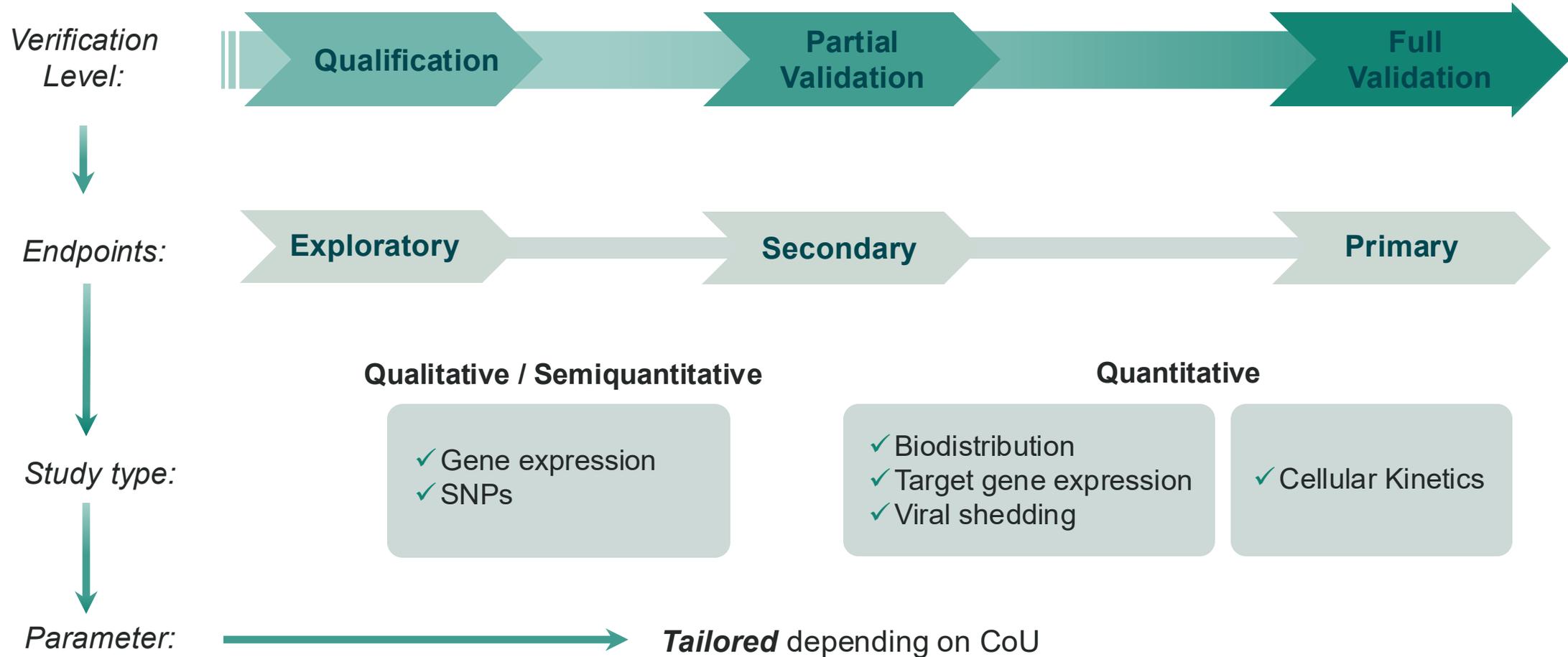
Validation Parameter	Qualitative	Semiquantitative	Quantitative
Accuracy	—	*(Optional)*	☑
Precision	☑	☑	☑
PCR efficiency	☑	☑	☑
Linearity	*(Optional: Input)*	*(Optional)*	☑
Sensitivity / LOB	—	☑	☑
Sensitivity / LOD	*The optimal input*	☑ *(Ct value, SD)*	☑
Sensitivity / LLOQ	—	—	☑ **(Absolute conc.)**
Specificity / Selectivity	☑	☑	☑
Stability	☑	☑	☑
Robustness	*(Optional)*	*(Optional)*	☑

Tailored depending on CoU

Internal experience

qPCR studies (2024-2025)

Pie chart:
- SNPs: 34%
- Gene Expression: 33%
- Quantification: 22%
- miRNA: 11%
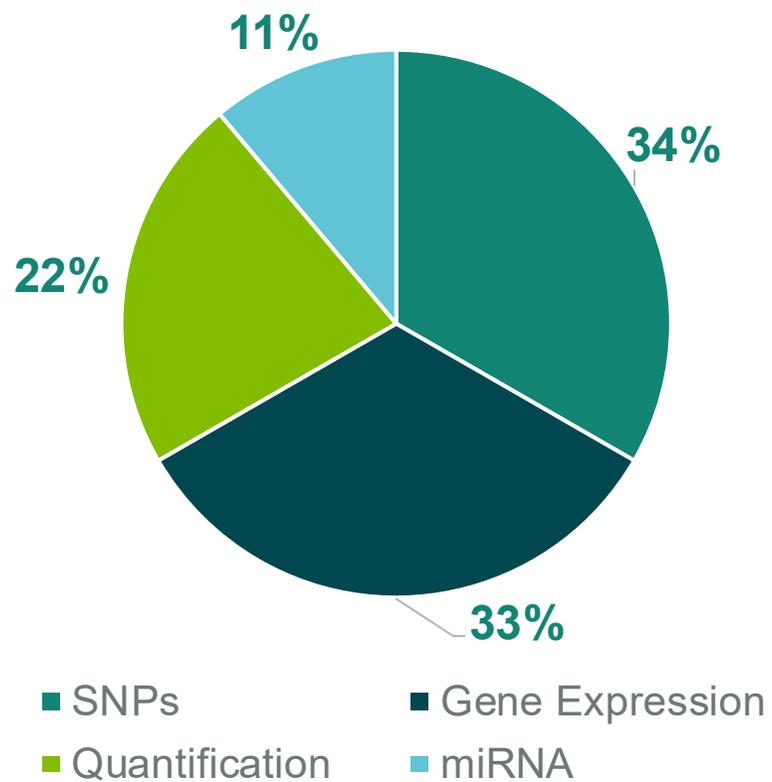
Samples	Qualitative	Semiquantitative	Quantitative
Whole blood	☑	☑	
Plasma/Serum			☑
Tissue: Liver, Kidney, Eye	☑	☑	
Urine			☑
Feces			☑

qPCR studies (2024-2025)

Validation Parameter	Gene Expression	miRNA	SNPs	Quantification
Accuracy				✅
Precision	✅	✅	✅	✅
PCR efficiency	✅	✅	✅	✅
Linearity	*Input*	✅	*Input*	✅
Sensitivity / LOB				✅
Sensitivity / LOD	*Optimal input*	✅ *(Ct value, SD)*	*Optimal input*	✅
Sensitivity / LLOQ				✅ *(Absolute conc.)*
Specificity			✅	✅
Stability	✅	✅	✅	✅
Robustness			*(Optional)*	✅

Extra-Parameters: Extraction efficiency

Yield	✅
Purity	✅
Integrity	*Optional*
Interference	*Optional*

Extra-Parameters:

CoU	✅

Practical Guidance

Bioanalytical phase

Method Development	Method Validation	Sample Analysis
Develop Method	Qualify or Validate the Method	Perform Sample Analysis
Confirmation of Amplificons	Report Qualification or Validation Result	Report Sample Analysis
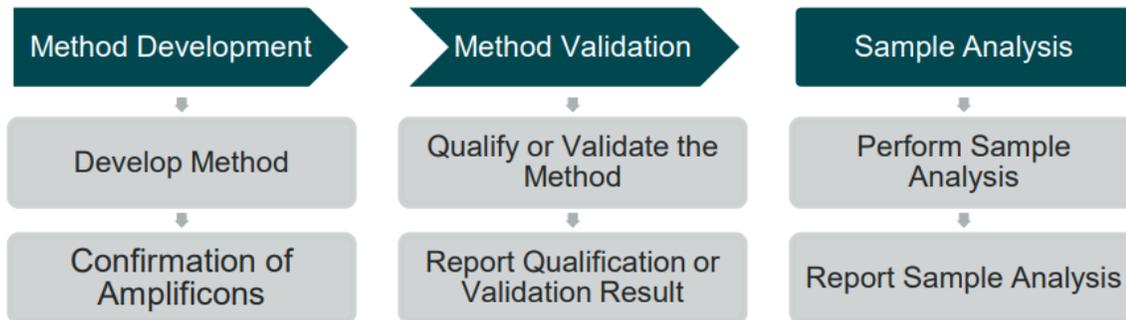
Focus in (on)

- Emphasize fit-for-purpose validation
- Match validation rigor to intended context of use
- Focus on scientific justification

Steps

1. Determine level of validation/qualification

2. Determine Assay Approach

3. Define assay materials, extraction methods

4. Define acceptance criteria and reportable without overextending LBA criteria

Don't forget:

1. GLP-compliant
2. Ensure documentation aligns with regulatory expectations

Key Takeaways

Rethinking validation is a challenge:
Refinement of White papers

We propose a practical, fit-for-purpose approach that balances scientific rigor while being tailored to the specific context of use

Focus on (in) CoU, Endpoints, Assay type (qualitative vs quantitative)