Making the Medicines of Tomorrow

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Platform method applications – leveraging knowledge and addressing challenges

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Lonza - your biologics end-to-end integrated service partner

Extensive track-record along the value chain

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Discovery | **Development** | **Market**

Basic Research & Drug Discovery → **Preclinical Testing** → **Clinical Trials** → **Commercial Production** → **Life-Cycle Management**

Technologies	Early Development & IND	Clinical Resupplies	BLA	sBLA

Comprehensive biologics offerings for every need

- Candidate selection & optimization
- Early bioconjugation technology selection and evaluation
- Early formulatability screens
- Proven, scalable, flexible expression system in your lab and ours

- Cell line construction
- Bioconjugates drug substance and drug product
- Formulation development

- Process development, optimization & characterization

- Manufacture of drug substance and drug product
- Fill and finish

- Regulatory support across the lifecycle

>740
Large molecules[1] in 2024

>670
Pre-clinical and clinical large molecules[1]

>70
Commercial large molecules[1]

[1]Including mammalian, microbial, bioconjugates, drug product services and cell & gene therapy products developed/manufactured by Lonza CDMO services (personalized medicines are included in pre-clinical and clinical molecules only, early development services are included for pre-clinical molecules only)

Phase appropriate analytical strategies

Analytical methods (platform or product-specific) aligned to a phase appropriate control strategy

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Development

- High throughput screening of titre, aggregation, fragmentation, glycosylation and process related impurities

Characterization

- Detailed analysis of amino acid sequence and post translational modifications eg glycosylation, deamidation, oxidation etc

Release and/or Stability

- Activity / Potency
- Purity (HMW species, LMW species, charge)
- Impurities (HCP, Residual Leached Ligand, DNA)
- Full suite of compendial methods for general characteristics, particles and biosafety

Purity

Aggregation	Fragmentation	Charge Variants
• SEC • SEC-MALS • DLS	• Capillary Electrophoresis • CE-SDS • SDS-PAGE	• icIEF • IEX • IEF

Impurities

Host Cell Protein	Host Cell DNA	Residual Affinity Ligand
• ELISA • Western Blot • 2D DIGE	• qPCR	• ELISA

Activity / Potency

Surface Plasmon Resonance	Cell Based Assays
• Receptor binding affinity • IgG Fc binding affinity	• Cell activation assays • Cell killing assays (ADCC, CDC) • Cell proliferation assays • Cell-cell interaction assays

Antigen Binding ELISA

Enzyme Activity Assays

Characterisation

Primary Sequence
- Amino acid sequence verification
- N- and C-terminal sequencing
- Sequence variant identification
- Amino acid composition

Post Translational Modification
- Degradation e.g. deamidation, oxidation
- Glycosylation site mapping
- Glycosylation profiling
- Sialic acid content
- Monosaccharide composition
- Disulphide bond characterisation
- Free cysteine content

Intact / subunit LC-MS analysis

Higher Order Structure
- CD
- DSC
- Fluorescence

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Platform analytical methods

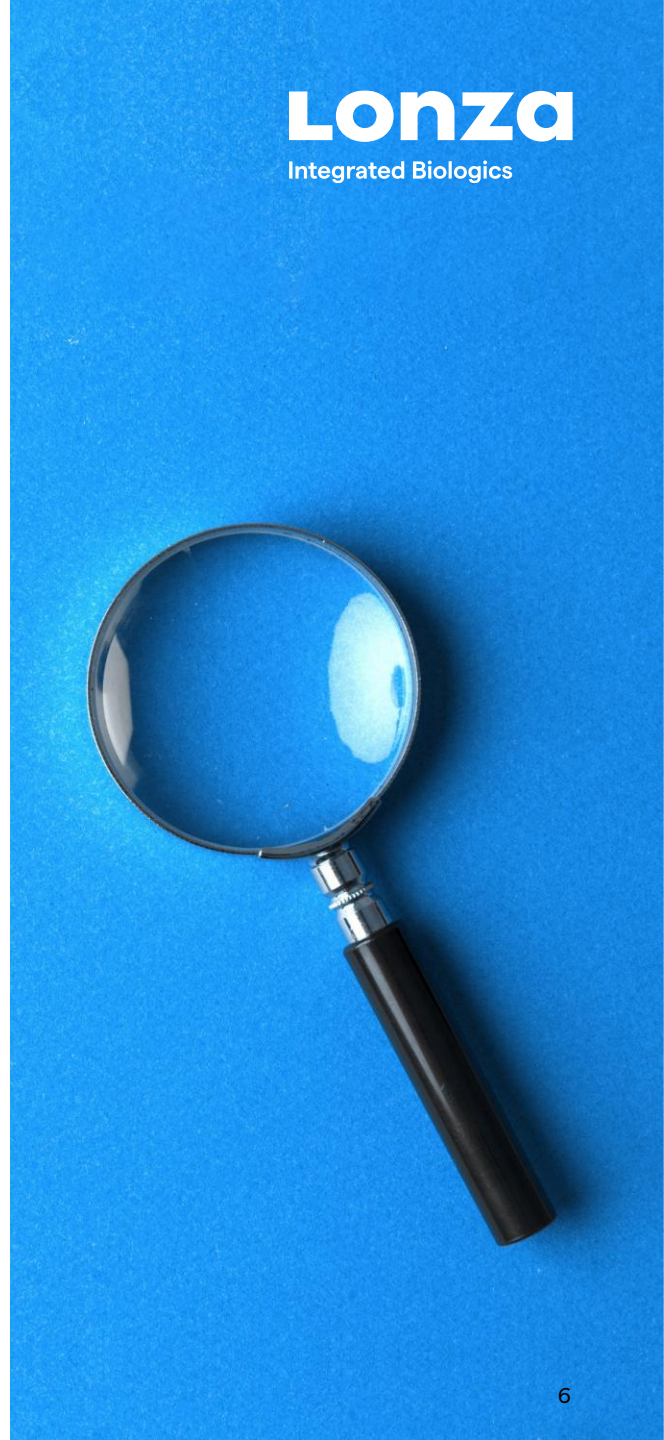
Definition & benefits

Definition of Platform Analytical Procedure (ICH Q2 (R)/ICH Q14):

*'An analytical procedure that is **suitable to test quality attributes of different products without significant change** to its operational conditions, system suitability and reporting structure. This type of analytical procedure can be used to analyse **molecules that are sufficiently alike** with respect to the attributes that the platform analytical procedure is intended to measure.'*

Benefits of Platform methods use include:

- Drives consistency and operational standardization across department and sites
- Allows faster and cost-effective development, leading to quicker time-to-clinic/market
- Established conditions allowing high reproducibility, easy execution and maintenance of quality standard
- Higher method knowledge and robustness



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Workflow of platform methods application

Phase appropriate approach

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Method development and validation

+

Establishment as platform method by conducting multi-product verification (platform bridging)

Platform methods library

Workflow of Platform Method application – phase appropriate approach

New molecule

01 Assessment if molecule qualifies for testing by platform methods

02 Rapid Platform method assessment

03 Platform method qualification

04 Method validation

Development

GMP testing

PPQ & commercial batch release

Workflow of platform methods application

Leveraging prior knowledge and data

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01 Assessment if molecule qualifies for testing by platform methods → **02** Rapid Platform method assessment → **03** Platform method qualification → **04** Method validation

Development | GMP testing | PPQ & commercial batch release

01. Assessment if molecule qualifies for testing by platform methods

From ICH Q14 (4.1 Knowledge Management):

"Prior **product knowledge** plays an important role in identifying suitable analytical techniques. **Knowledge** of best practices, state-of-the-art technologies and regulatory expectations contribute to the selection of the **most suitable technology** for a given purpose. **Existing platform analytical procedures can be leveraged to evaluate the attributes of a specific product without conducting additional procedure development."**

Theoretical assessment based on:

Molecule knowledge

- Molecular weight (MW)
- Affinity ligand binding
- Expected modifications
- Isoelectric point (pI)
- Manufacturability assessment

+

Key CQAs & prior data

- Size variants
- Product impurities
- Charge variants
- Glycosylation profile
- Process Impurities

+

Technology and platform methods knowledge

- SEC
- CE-SDS
- icIEF
- Small scale purification
- PrA HPLC
- ELISA

→ Selection of applicable platform methods based on prior knowledge

Platformability of different molecule types

Potential of Platform Methods to be suitable for testing

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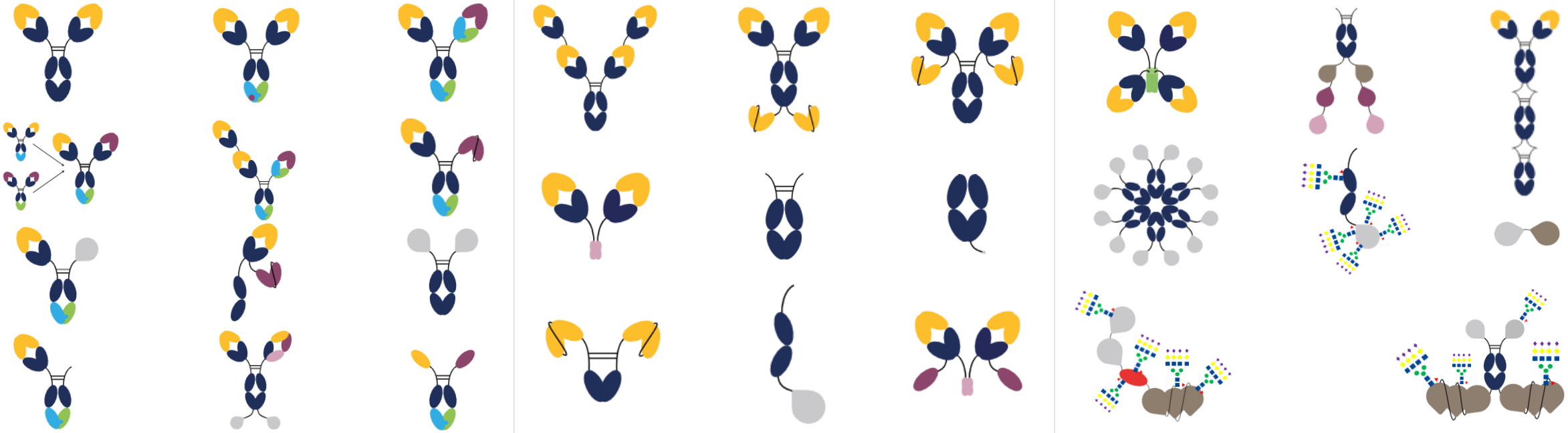
ICH Q14 '...This type of analytical procedure can be used to analyse molecules that are sufficiently alike...'

High	Moderate	Low



Platform methods suitability at Lonza goes beyond mAb/ mAb-like molecules

Platformability of different molecule types

High success rate of platform methods application to bispecifics and fc-fusion proteins DNA to IND programs

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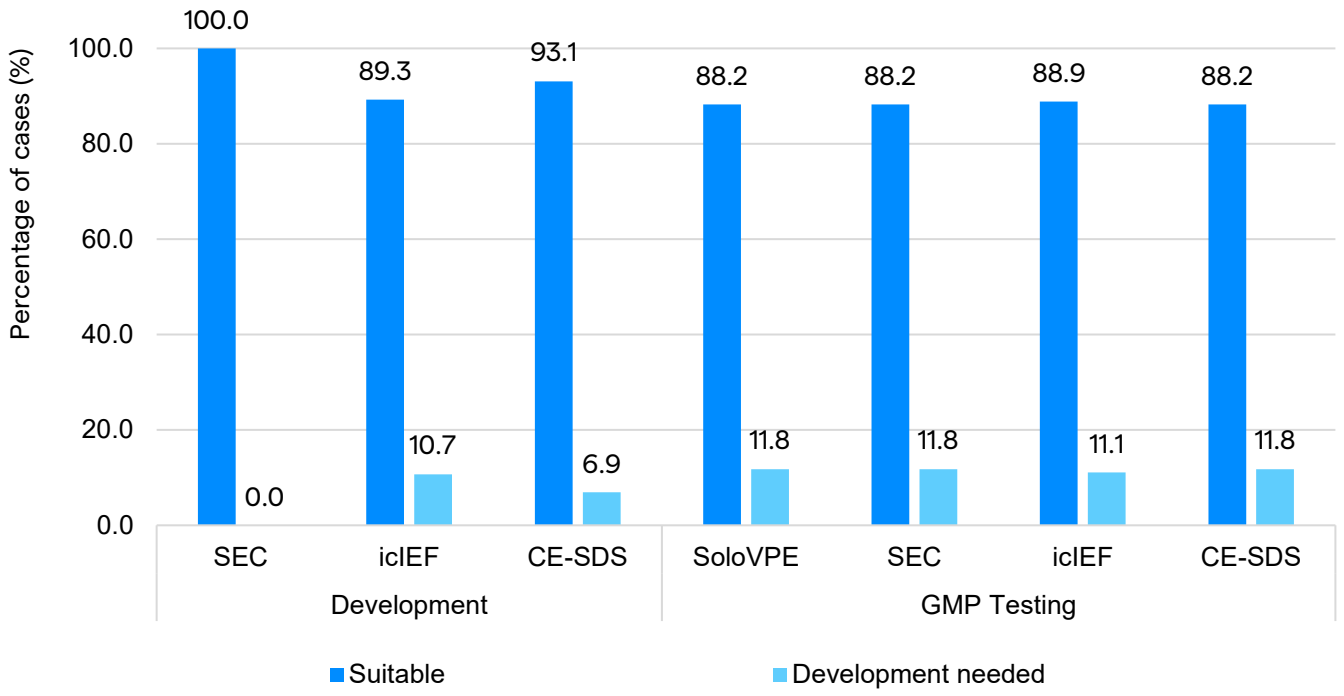
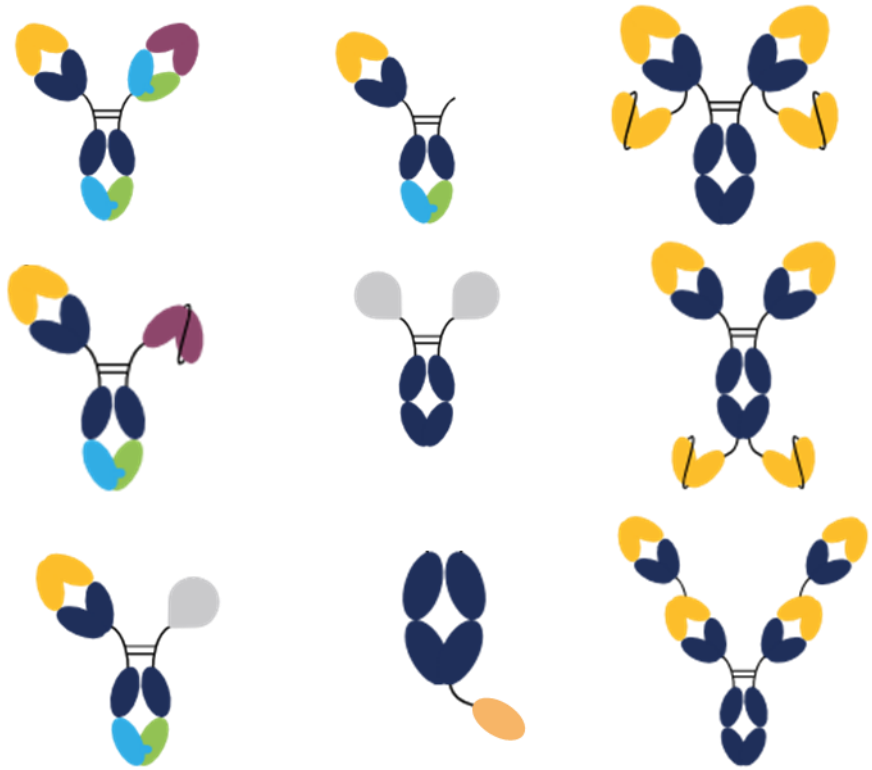
Based on a review of a range of Bispecifics and Fc-fusion proteins programs

Success Rate of Platform Methods application





Workflow of platform method application

Phase appropriate method assessment and validation

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02. Rapid Assessment of Platform methods to support Development stages

Performed for a subset of methods to:

- Evaluate the performance and suitability of selected platform methods for use in early development
- Make recommendations regarding development of product specific methods as appropriate.

As per ICH Q14, knowledge management: this step may not be required depending on previous experience and knowledge related to molecule type and platform method.
Usually performed when molecule features are outside expected range to fit platform methods, and there is not enough prior knowledge.

Workflow of platform method application

Phase appropriate method assessment and validation

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Phase appropriate validation for a specific product

03
Platform Method Qualification

04
Method Validation

The scope of these steps can be reduced based on prior knowledge and data obtained during platform development and validation studies, especially when using ICH Q14 enhanced approach.
ICH Q14: "...For a new application of such platform analytical procedures, the subsequent development can be abbreviated, and certain validation tests can be omitted based on a science- and risk-based justification..."

Case study #1 - platform methods application

Bispecific antibody DNA to IND program using platform methods

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01 Assessment if molecule qualifies for testing by platform methods

02 Rapid Platform method assessment

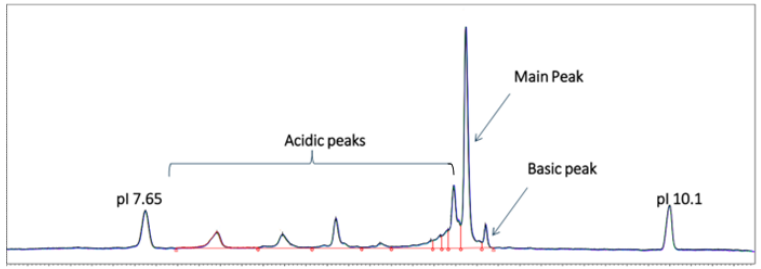
03 Platform method qualification

Development

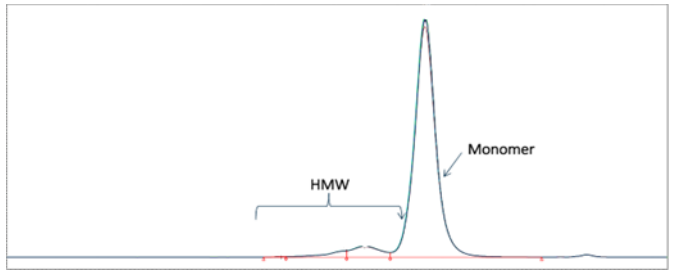
GMP testing

Three stage process followed: all platform methods suitable for both Development stage and GMP Testing

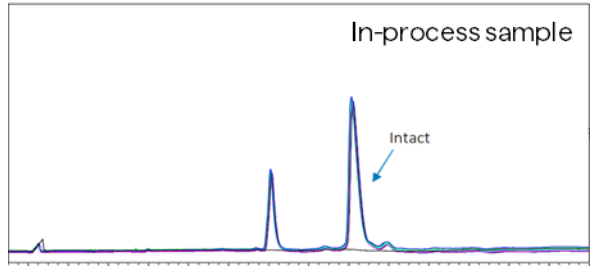
IcIEF platform method*



SEC platform method*



Non-reduced CE-SDS platform method*



- SoloVPE
- Impurity methods

*Repeatability results: overlay of 6 individual preparations

Case study #2 - platform methods application

Heterodimeric multichain bispecific molecule (IgG1) DNA to IND program

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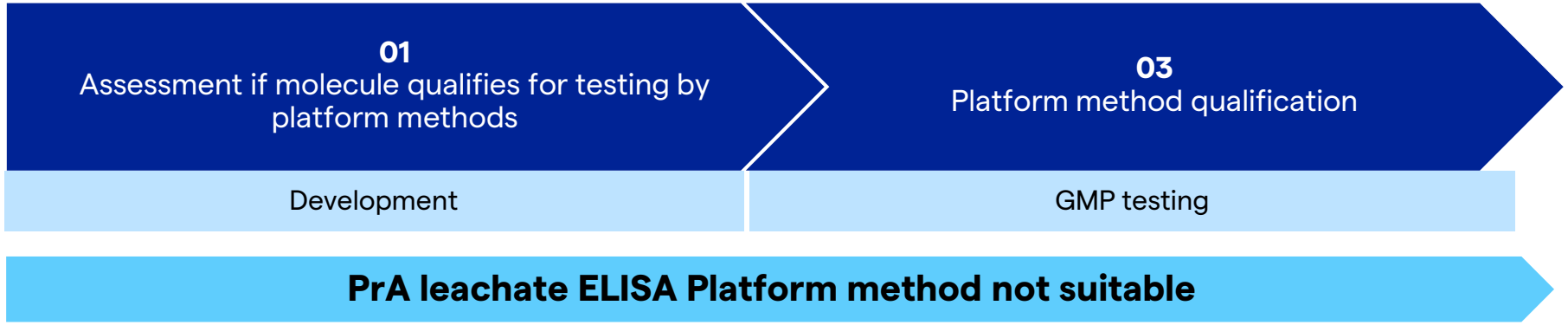

Most platform methods suitable for both Development stage and GMP Testing, <u>except PrA leachate ELISA</u>

- Platform Impurity Methods – usually assumed suitability for most molecules and processes based on prior knowledge, and experimental method establishment assessment (step 2) not performed.
- The dilution tested for the product as per the platform method **failed to meet acceptance criteria**
- Root cause: DP or other leachates interfering with platform method – platform method not suitable
- Recommendation: **development of a product-specific method using alternative kits**

Concentration (mg/mL)	Theoretical spike concentration	Spike recovery range	Number of samples within acceptable recovery range
0.500	7 concentrations in the range 0.125-8 ng/mL	30-64%	0 (none)
0.250		38-88%	1
0.125		52-96%	4
0.063		63-124%	5
0.031		64-107%	6
0.016		74-120%	7 (all)

Case Study #2 - product specific PrA ELISA method development

Heterodimeric multichain bispecific molecule (IgG1) DNA to IND program

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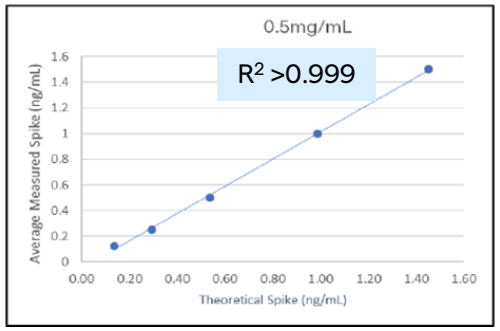
Development of a product-specific PrA ELISA method using alternative commercial kit

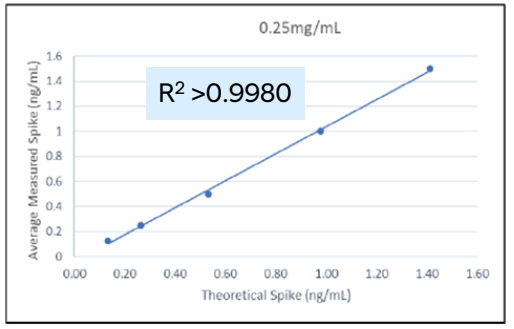
Acceptable recoveries observed for sample dilution in the range 0.075-0.5 mg/mL for all the spike levels tested

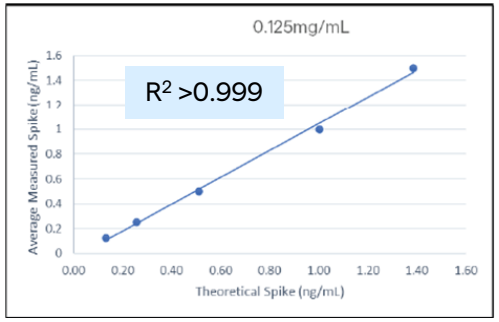
Concentration (mg/mL)	Theoretical Spike concentration	Spike recovery range
0.500	5 concentrations in the range 0.125-1.5 ng/mL	72-81%
0.250		72-82%
0.125		72-81%
0.100		76-88%
0.075		73-80%

Successful development and qualification of a PrA ELISA test method, confirmed to be suitable for relative quantitation of residual PrA

Acceptable linearity at different product concentrations







Rapid method development for non-platform molecules

Using analytical toolbox and ICH Q14 enhanced approach elements

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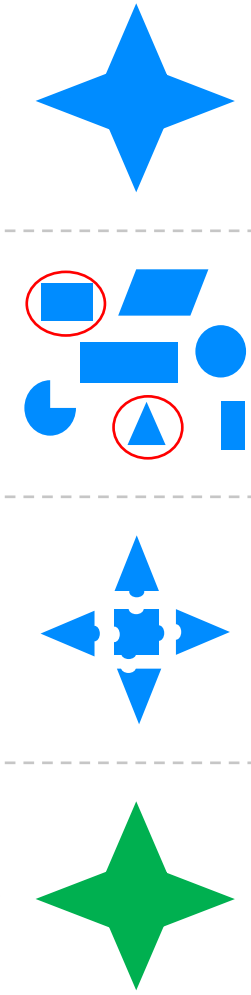
Application of extensive analytical toolbox combined with analytical development experience to quickly develop methods to measure product quality attributes for different molecule formats

- Define target molecule CQAs and Analytical Target Profile (ATP) as applicable (QbD elements)
- Select pre-defined start point(s) from analytical toolbox (instead of method development from scratch)
- Develop and optimise analytical method for target molecule, using OFAT or DOE approach
- Phase appropriate validation



Case Study #3 – RP HPLC method development

Heterodimeric multichain bispecific molecule (IgG1) DNA to IND program

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No suitable platform method available for HT screening of chain pairing variants during cell line development

Product-specific RP HPLC method development performed, using analytical toolbox

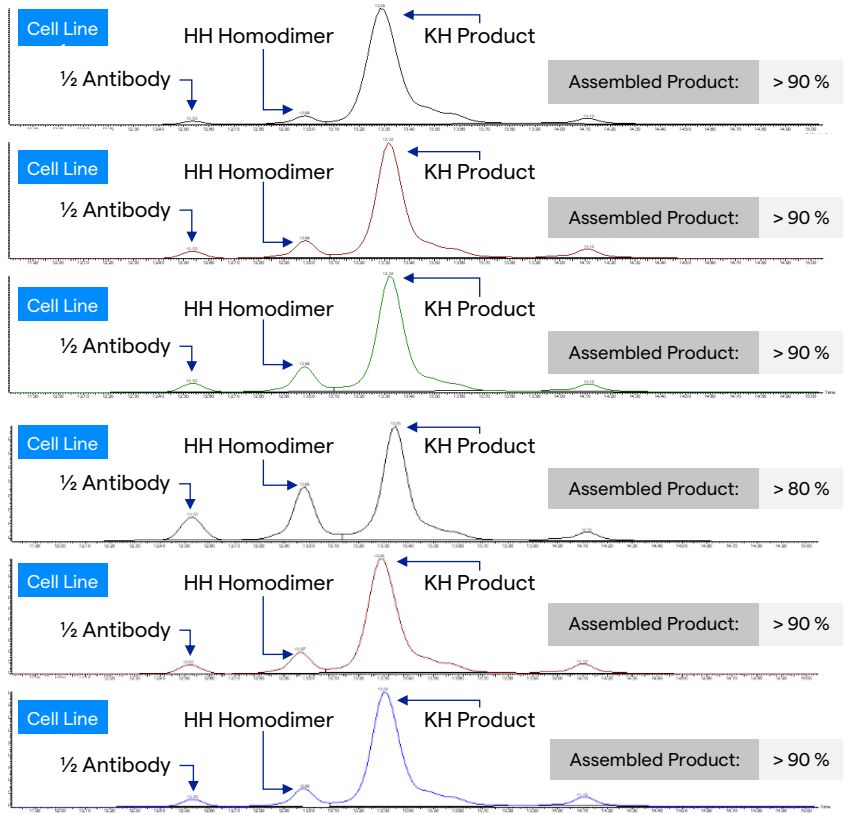
Analytical toolboxes covering starting point RP HPLC method and method optimisation approach were applied

Development of the RP HPLC method in approximately 3 weeks from the receipt of early partially purified material

The developed RP HPLC conditions were used to screen cell lines for relative levels of homodimer formation

Method capable of supporting screening of 48 cell lines with verified results available within 5 days of testing initiation

RP HPLC testing alongside affinity-based titre, SEC, chip electrophoresis and glycan profiling methods allowed us to identify cell lines that met both titre and product quality requirements for the program



Example data from cell line development work presented

Platform methods application - case study #5

Non-Fc protein, highly Glycosylated and Phosphorylated - DNA to IND program

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Most platform methods not suitable for both Development stage and GMP Testing

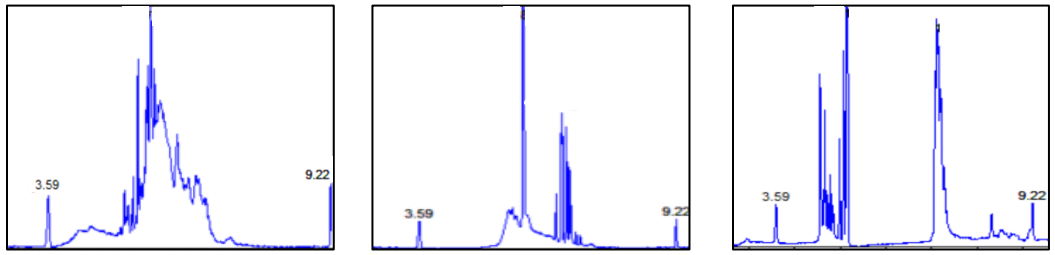
Method development required for several CQAs including titer, charge variants, fragments, leachate impurities

Glycan and aggregation analysis performed using platform methods – validation required to extend platform method application to this class of molecule

Product-specific AEX method development performed, using analytical toolbox

IclEF Profile

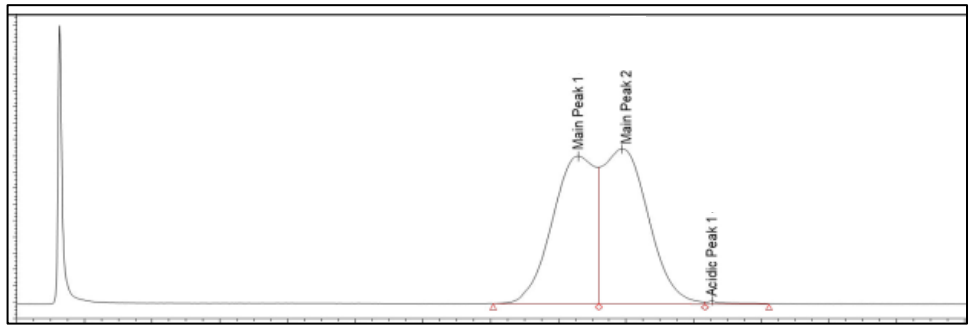
Intact molecule	Desialylated molecule	Dephosphorylated and desialylated molecule



- iclEF peaks were not well resolved
- Phosphatase peak interfered with the product peak

AEX Method development using analytical toolbox

Developed AEX Method



Dephosphorylated molecule

Successful development and validation of an AEX test method, confirmed to be suitable for charge variants analysis

Leveraging ICH Q14 enhanced approach for development of platform methods

Robust platform methods applied to broader range of molecules

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Benefits

- Better understanding of the impact of analytical procedure parameters on the method performance
- Wider operating ranges, within method operable design region (MODR), that fulfils method performance
- Allows platform method readiness for a broader range of products
- Ensures robustness and more flexibility for regulatory and lifecycle management
- More flexibility to method development and validation, and reduces replication of validation steps between phases

Operational challenge

- Defining an efficient QbD framework that can easily be implemented, while guaranteeing time to clinic and allowing shorter subsequence method validation stages.

Could enhanced approach facilitate achievement of compendial performance for platform methods, eliminating even further the need for validation steps within different phases?

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Conclusions and take-home message

Platform methods and ICH Q14

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- Platform methods have several benefits, including **operational standardization, faster and cost-effective** development
- The workflow for platform method application should consider a **phase appropriate** method assessment and validation
- The **scope** of platform method assessment and **validation can be reduced based on prior knowledge** and data obtained during platform development and validation studies
- Platform methods **application can be expanded to a range of molecule types**, although they are not a universal fit, and **molecule specific challenges** need to be addressed through product specific method development
- Rapid and flexible analytical method development can be achieved by using advanced **analytical toolbox and QbD elements** as starting points
- Application of **ICH Q14 enhanced approach** for overall method development ensures **robustness and flexibility** throughout lifecycle management
- Leveraging enhanced approach for development of platform methods can led to application to a broader range of molecules, more predictability, reduced risks and speed to clinic. But implementation of an efficient QbD framework for method development has its challenges

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