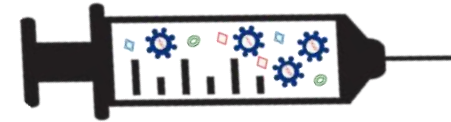


Applied AQbD from Concept to Control

A Practical ICH Q14 Implementation Case Study Using
Capillary Electrophoresis for Rapid Virus Quantification



Dr. Ewoud van Tricht, Prof. Dr. Cari Sänger - van de Griend

Co-owner and principal consultant, **Kantisto BV**

Scientific director, **BioQC Ltd**

www.kantisto.nl

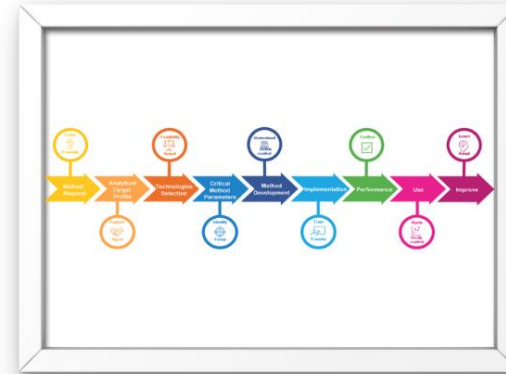
www.bio-qc.com



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BioQC
Quality Knowledge

2
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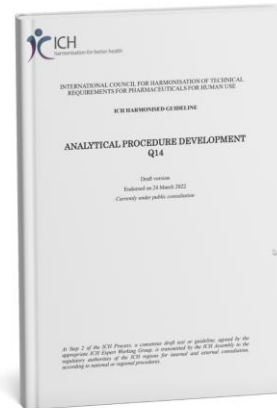


Guidelines related to analytical development and validation

Analytical Quality by Design (AQbD)

ICH Q14

Analytical Procedure Development



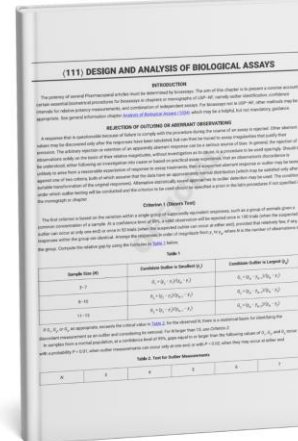
Draft USP <1220>

Analytical Procedure Life Cycle



USP <111>

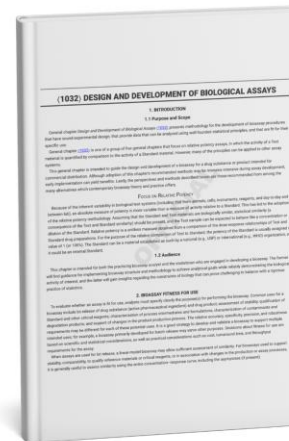
Design and Analysis of Biological Assays



Bioassays

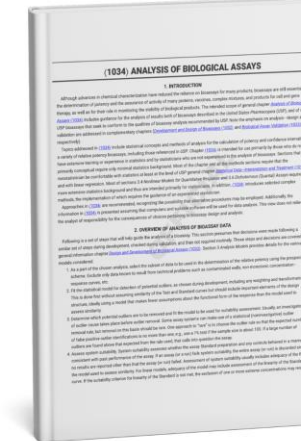
USP <1032>

Design and Development of Biological Assays



USP <1034>

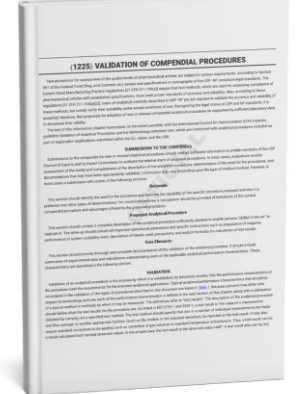
Analysis of Biological Assays



Compendial

USP <1225>

Validation of Compendial Procedures



Analytical procedures

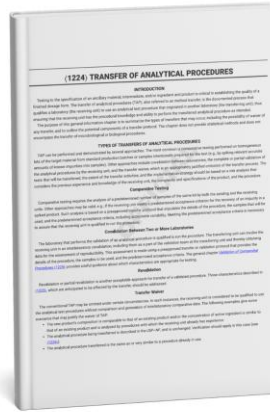
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Analytical Data – interpretation and treatment



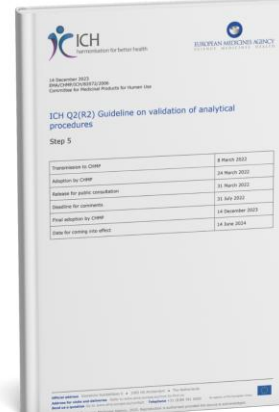
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Transfer of Analytical Procedures



ICH Q2(R2)

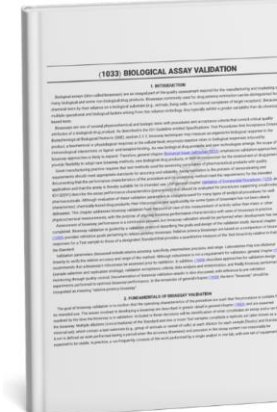
Guideline on Validation of Analytical Procedures



Validation

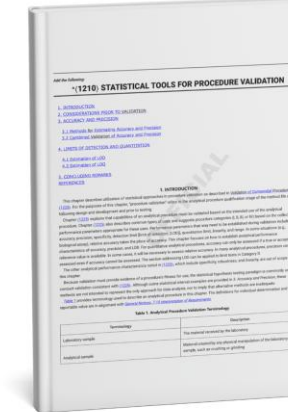
USP <1033>

Biological Assay Validation



USP <1210>

Statistical Tools for Procedure Validation

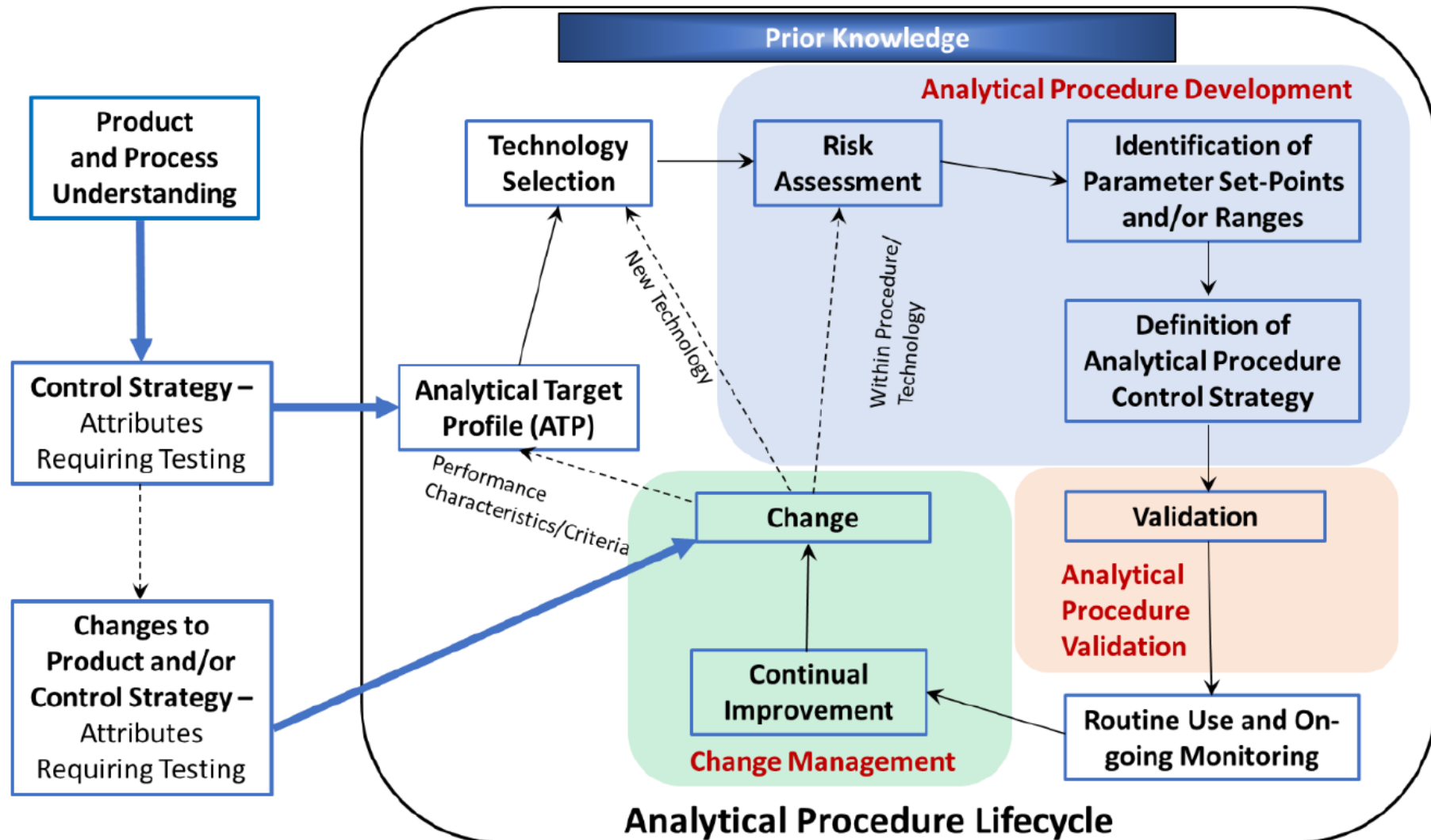


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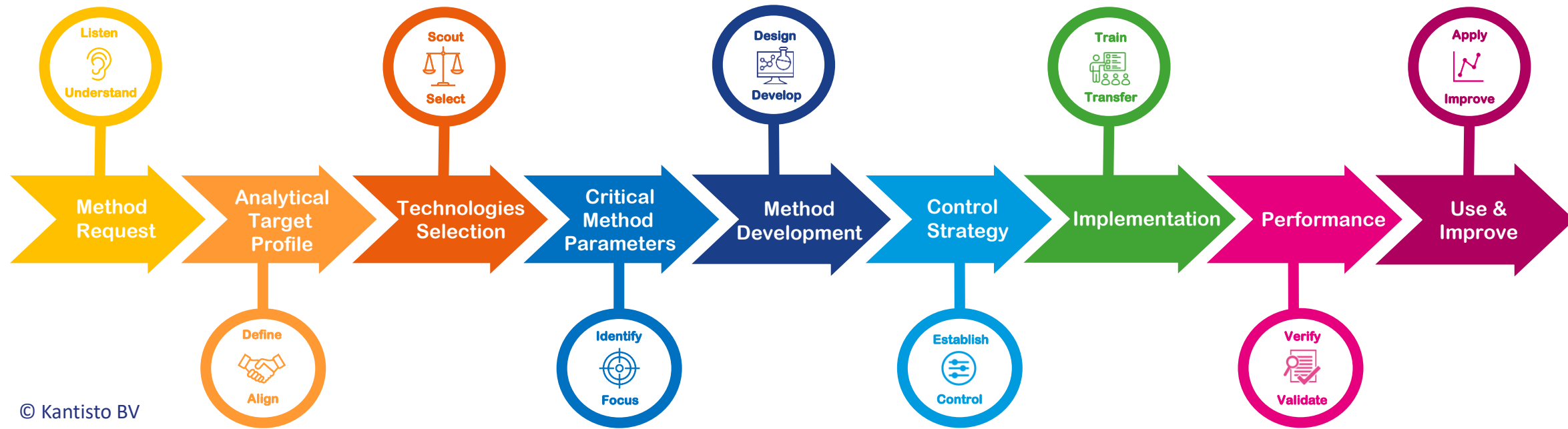
Verification of Compendial Procedures



ICH Q14 Analytical Procedure Lifecycle

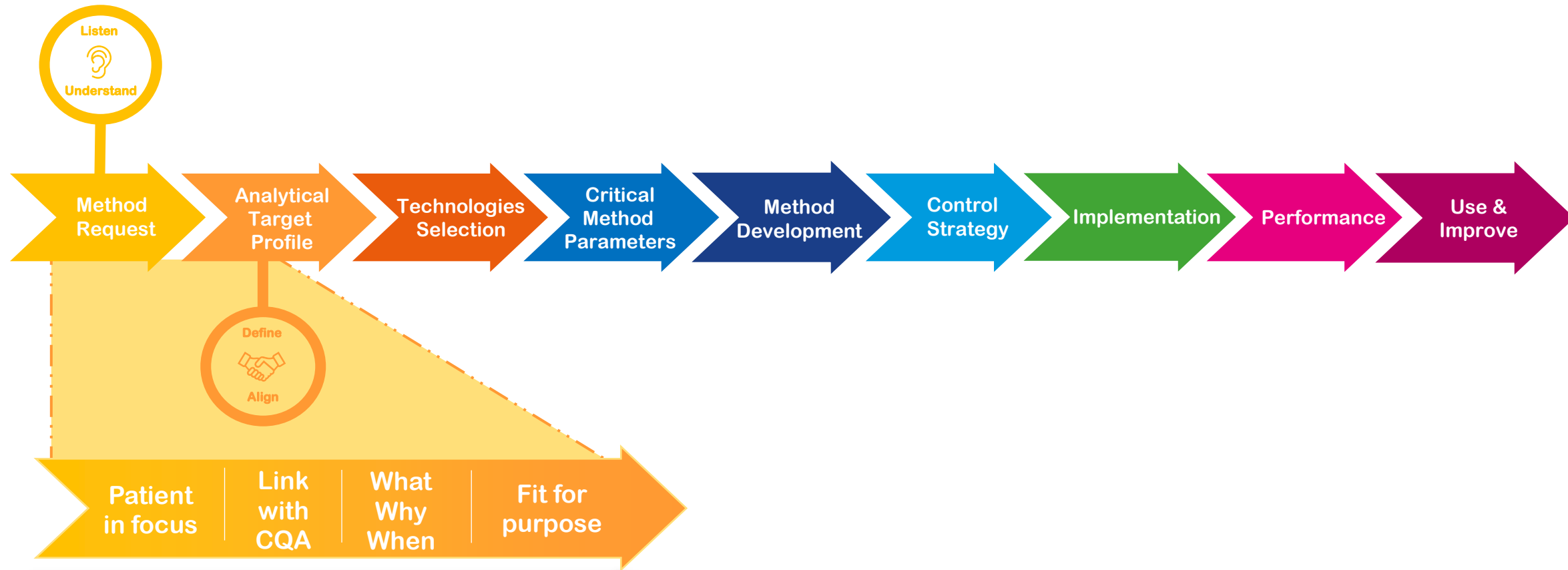


Our AQbD approach



© Kantisto BV

Our AQbD approach - Method request and ATP



Method Request

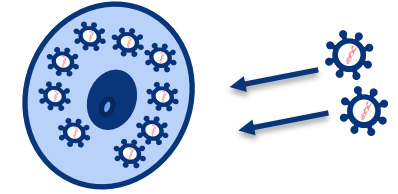
Adenoviruses

- 90 – 100 nm diameter
- Total M_w : 150 MDa
(1000 times larger than antibody)
- Core: DNA

Production process

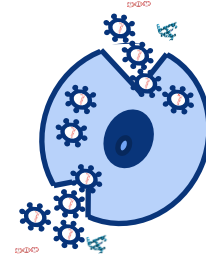
Upstream processing (USP)

Cell growth and virus replication

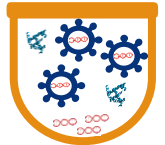


Down stream processing (DSP)

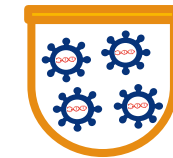
Cell lysis
Release of virus



Clarification and
DNA precipitation



Anion exchange
chromatography



Diafiltration and
ultrafiltration

Final product

Formulation

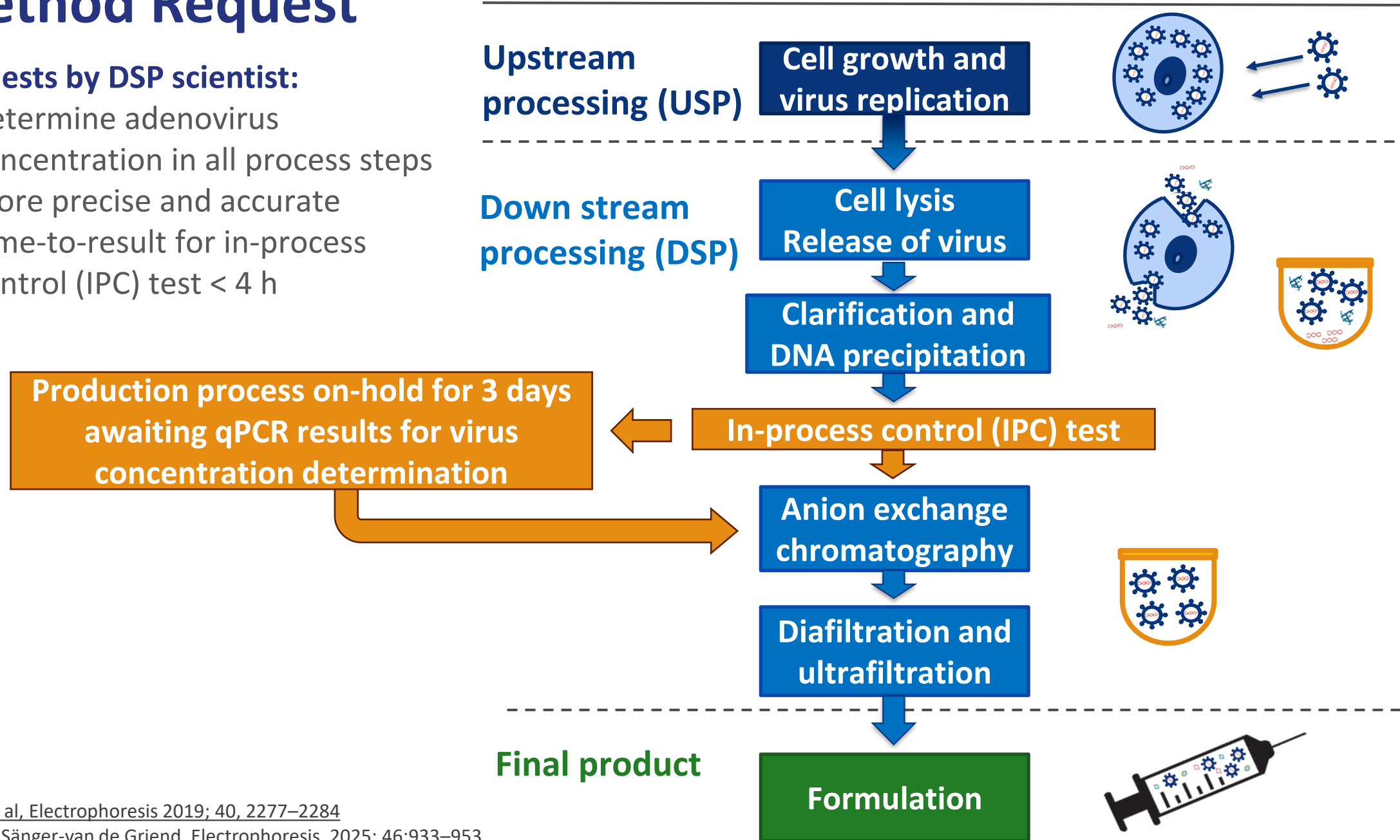


Method Request

Requests by DSP scientist:

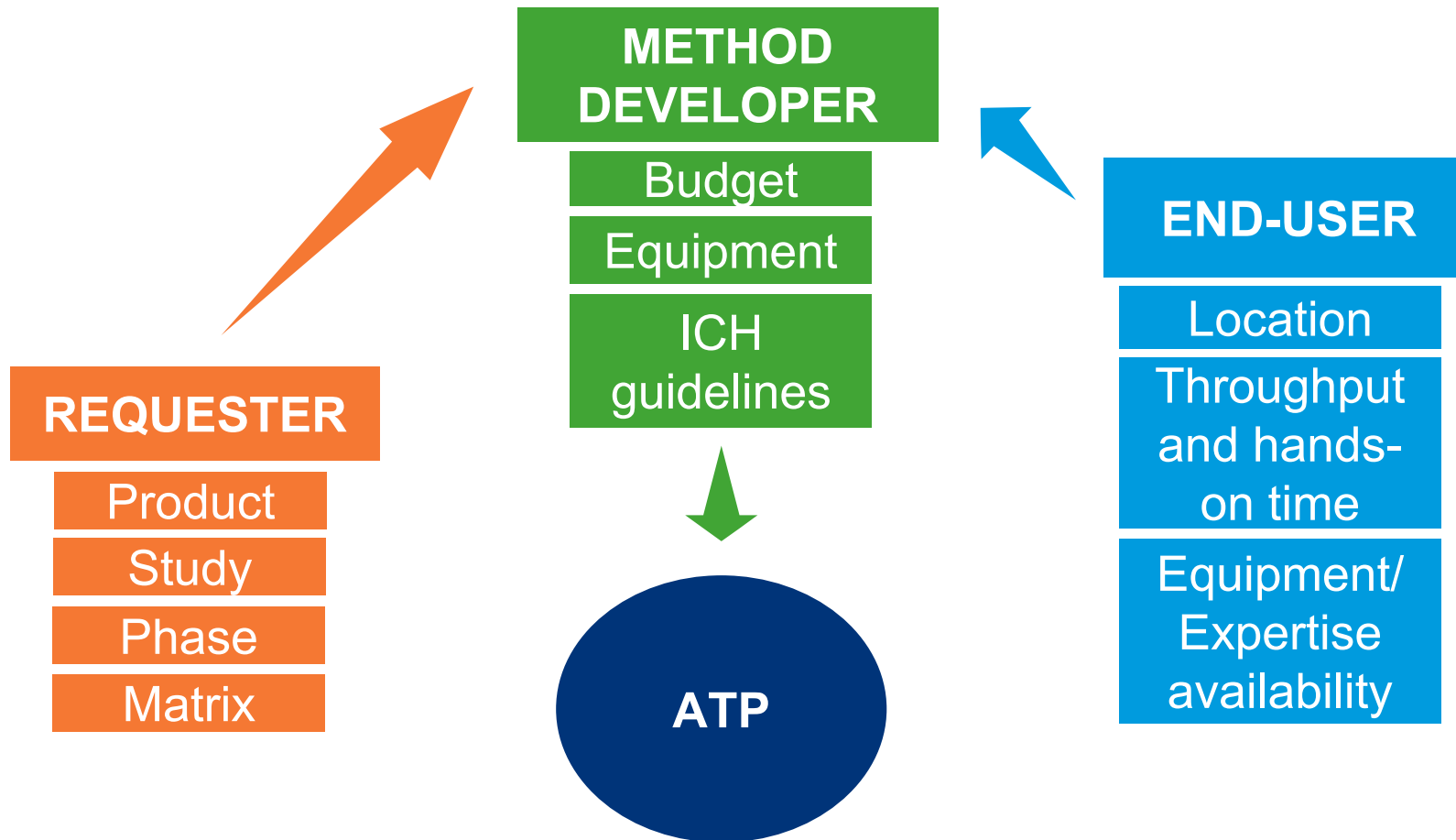
- Determine adenovirus concentration in all process steps
- More precise and accurate
- Time-to-result for in-process control (IPC) test < 4 h

Production process



The Analytical Target Profile (ATP)

The ATP is a list of analytical and business requirements from the **requester**, **method developer**, **end-user**, and the possible **future end-user**



The Analytical Target Profile (ATP)

REQUESTOR

METHOD DEVELOPER

END-USERS

Summary of request		Priority	Targets and ranges	
Product quality attribute			Virus particle concentration determination (in VP/ml)	
Clinical phase			Phase I – commercial	
Aim of study/method			1) For adenovirus production process development and product characterization a precise, accurate, robust, degradation sensitive, and a high-throughput method is needed for the quantitation of adenovirus particles in all process intermediates, DS, and DP.	2) An accurate adenovirus amount should be loaded upon the AEX- filters, to avoid filter blockage, excessive viral dilution, and impurity break through and to avoid product degradation whilst testing, a precise, accurate, robust, and fast method for adenovirus quantitation is required for in-process control testing (IPC).
Product description and/or process step		1	Crude harvest, Lysed harvest, Clarified harvest (IPC), Anion exchange product, Diafiltration product, Drug substance, Drug product	
Analyte(s)		1	Adenovirus type 26 and type 35	
Matrices			The matrix could contain dell derbis, residual DNA, salt, protein and or formulation buffer components dependent on the process intermediate	
Concentration range(s)		3	10 ¹⁰ – 10 ¹² VP/ml (~ 170 pmol/L)	
Requester requirements	Time-to-result	2	< 2 days for process development (1), < 4 h for IPC testing (2)	
Type of test			Content / potency	
Required validation status			Qualified	
ICH Q2-characteristics	Specificity		Detection of Ad26 and Ad35 without significant interference of sample matrix components	
	Range / linearity	3	1·10 ¹⁰ – 1·10 ¹² VP/ml	
	Accuracy	3	≤ 10% bias	
	Precision / Intermediate Precision	2	Intermediate precision ≤ 10% RSD	
End-user			Quality control	
End-user requirements	Throughput	3	1 sample per run for IPC testing, > 7 samples per process development run. > 300 samples a month for process development.	

The Analytical Target Profile (ATP)

REQUESTOR

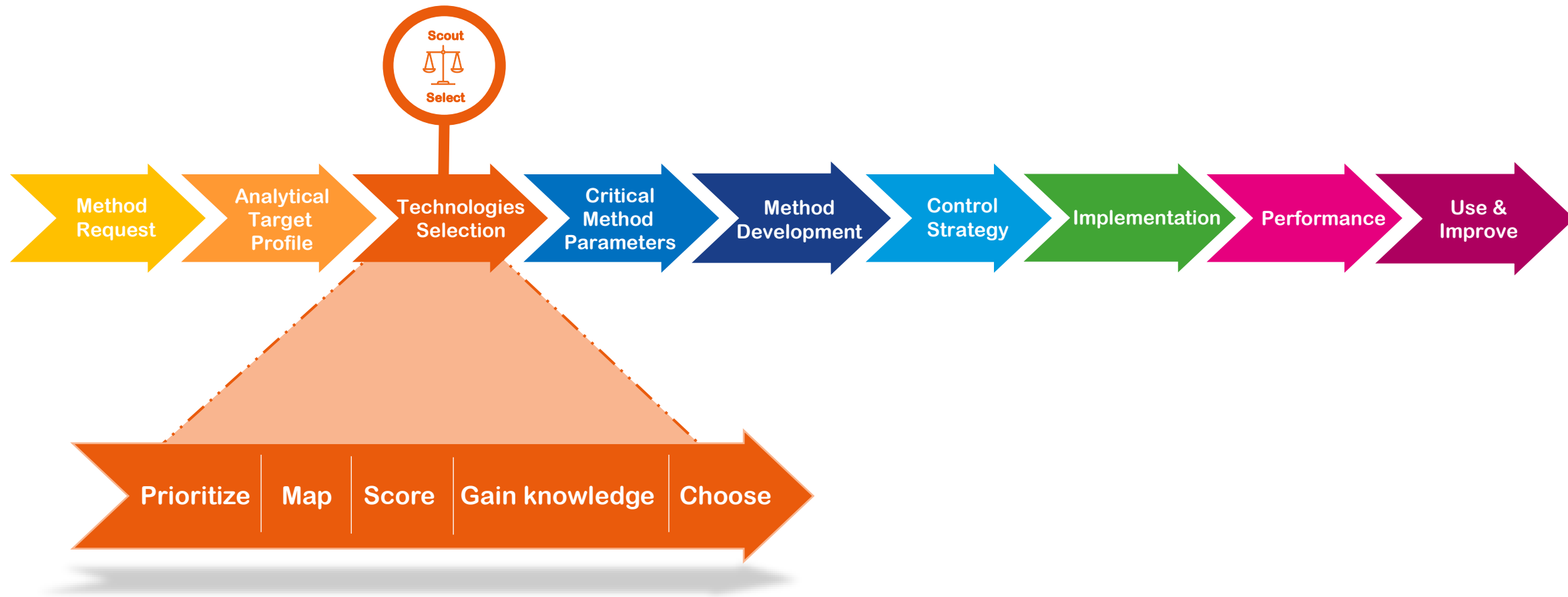
METHOD DEVELOPER

END-USERS

Summary of request		Priority	Targets and ranges	
Product quality attribute			Virus particle concentration determination (in VP/ml)	
Clinical phase			Phase I – commercial	
Aim of study/method			1) For adenovirus production process development and product characterization a precise, accurate,	2) An accurate adenovirus amount should be loaded upon the AEX- filters, to avoid filter blockage, excessive and impurity break through and to avoid
			tion whilst testing, a precise, accurate, method for adenovirus quantitation is process control testing (IPC).	
			duct, Diafiltration product, Drug	
			ormulation buffer components	
			matrix components	
Product description and/or p				
Analyte(s)				
Matrices				
Concentration range(s)				
Requester requirements				
Type of test				
Required validation status				
ICH Q2-characteristics	Accuracy	3	≤ 10% bias	
	Precision / Intermediate Precision	2	Intermediate precison ≤10% RSD	
End-user			Quality control	
End-user requirements	Throughput	3	1 sample per run for IPC testing, > 7 samples per process development run. > 300 samples a month for process development.	



Our AQbD approach - Technologies selection



Technologies selection

Select optimal techniques to adhere to ATP requirements and business urgency (priority and impact).

			Priority	Techniques							
				OD260	AEX-LC-UV	RP-UPLC-UV	SEC-LC	qPCR	CE-UV	Virocyt	AF4-UV
Requester	Requirements		Virus particle concentration determination (in VP/ml)								
	Process intermediate(s) and matrix		Crude harvest, Lysed harvest, Clarified harvest (IPC), Anion exchange product, Diafiltration product, Drug substance, Drug product. The matrix could contain dell derbis, residual DNA, salt, protein and or formulation buffer components dependent on the process intermediate	1	Only DS/DP	Only DS/DP	All	Only DS/DP	All	All	Only DS/DP
Requester requirements	Time-to-result	< 4 h for IPC testing	2	< 4 h	4 h	4 h	4 h	4 h	1 h	1 h	4 h
Method developer	Specificity	Detection of Ad26 and Ad35 without significant interference of sample matrix components	1	Aromatic peptides and nucleotides	Adenovirus particles	Adenovirus protein	Adenovirus particles and protein aggregation	Adenovirus encapsulated DNA	Adenovirus particles	Adenovirus DNA and protein epitope	Adenovirus particles
		Accuracy (spiked recovery)	2	80 - 120%	80 - 120%	75 - 125%	80 - 120%	70 - 130%	90 -110%	Unknown	80 -120%
	Precision	Intermediate precision ≤10% RSD	2	20%	10 - 20%	10 - 20%	10 - 20%	10 - 35%	5%	9% (uncertain)	20%
	Concentration range	10 ¹⁰ - 10 ¹² VP/ml	3	> 10 ¹⁰ VP/ml	> 10 ¹⁰ VP/ml	> 10 ¹⁰ VP/ml	> 10 ¹⁰ VP/ml	> 10 ⁹ VP/ml	> 10 ¹⁰ VP/ml	> 10 ⁷ VP/ml (uncertain)	> 10 ¹⁰ VP/ml
	End-user	Quality control		Yes	Yes	Yes	Yes	Yes	Yes	Maybe	No
End-user requirements	Throughput	1 sample per run for IPC testing, > 7 samples per process development run. > 300 samples a month for process development.	2	> 300	300	300	300	< 300	> 300	> 300	300
	Equipment availability	Yes		Available	Available	Available	Available	Available	Available	Not available	Not in QC

STEP 1: Prioritize the ATP requirement

STEP 2: Map all possible technologies

STEP 3: List estimated figures of merit

STEP 4: Colour scoring based on risk (high, medium, low)

STEP 5: Select 1 or more technologies based on risks and timelines

Technique of Choice:
Capillary Electrophoresis
with UV detection

Technologies selection

Select optimal techniques to adhere to ATP requirements and business urgency (priority and impact).

Requester	Requirements	
	Process intermediate(s) and matrix	
Method developer	Requester requirements	Time-to-result
		Specificity
		Accuracy
		Precision
End-user	End-user	
	End-user requirements	Throughput
	Equipment availability	

The right technology for the right purpose



Techniques

qPCR	CE-UV	Virocyt	AF4-UV
All	All	All	Only DS/DP
4 h	1 h	1 h	4 h
Adenovirus capsulated DNA	Adenovirus particles	Adenovirus DNA and protein epitope	Adenovirus particles
100 - 130%	90 - 110%	Unknown	80 - 120%
100 - 35%	5%	9% (uncertain)	20%
10 ⁹ VP/ml	> 10 ¹⁰ VP/ml	> 10 ⁷ VP/ml (uncertain)	> 10 ¹⁰ VP/ml
Yes	Yes	Maybe	No
< 300	> 300	> 300	300
available	Available	Not available	Not in QC

Technique of Choice:

Capillary Electrophoresis with UV detection

STEP 2: Map all possible technologies

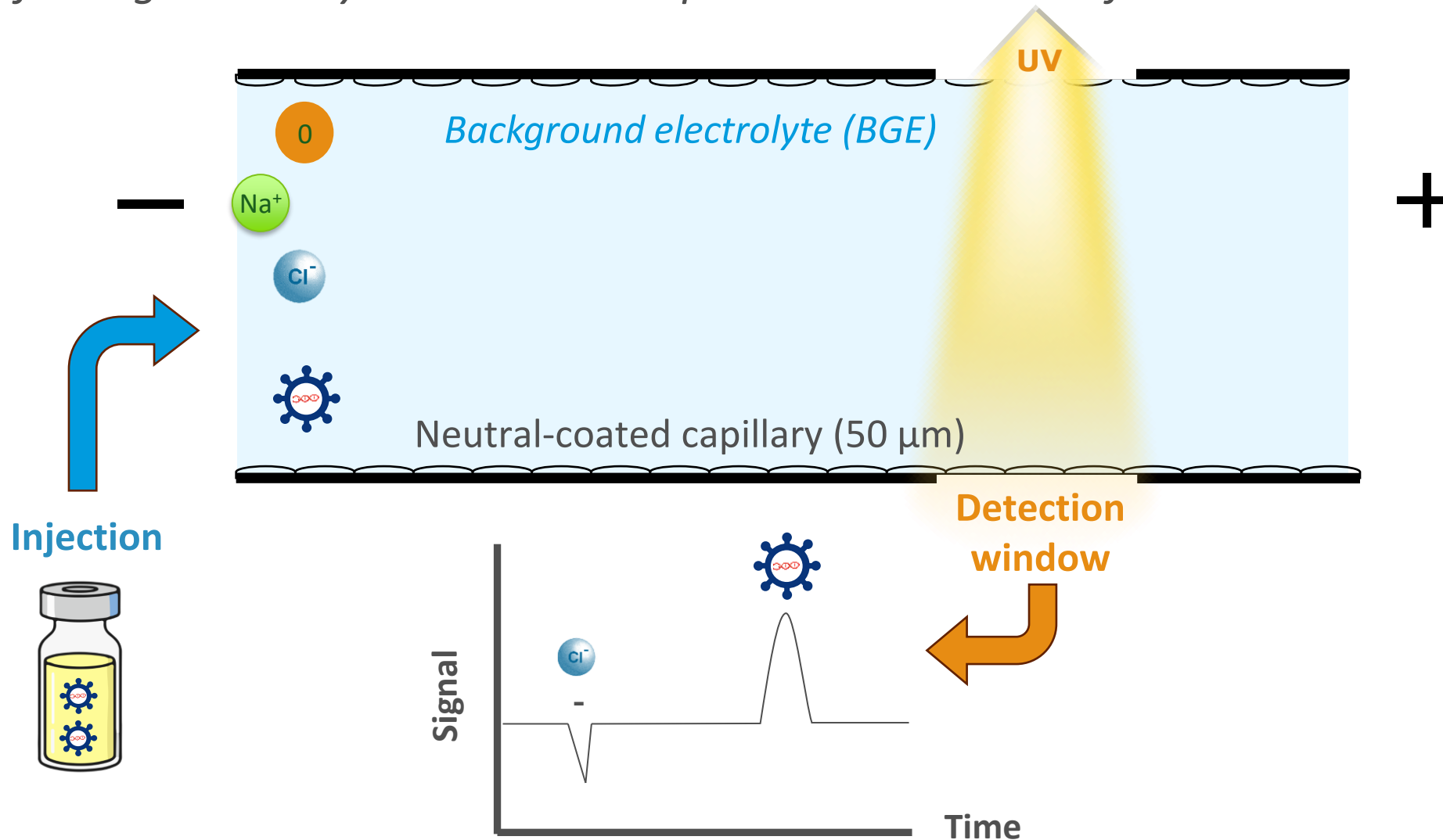
STEP 3: List estimated figures of merit

STEP 4: Colour scoring based on risk (high, medium, low)

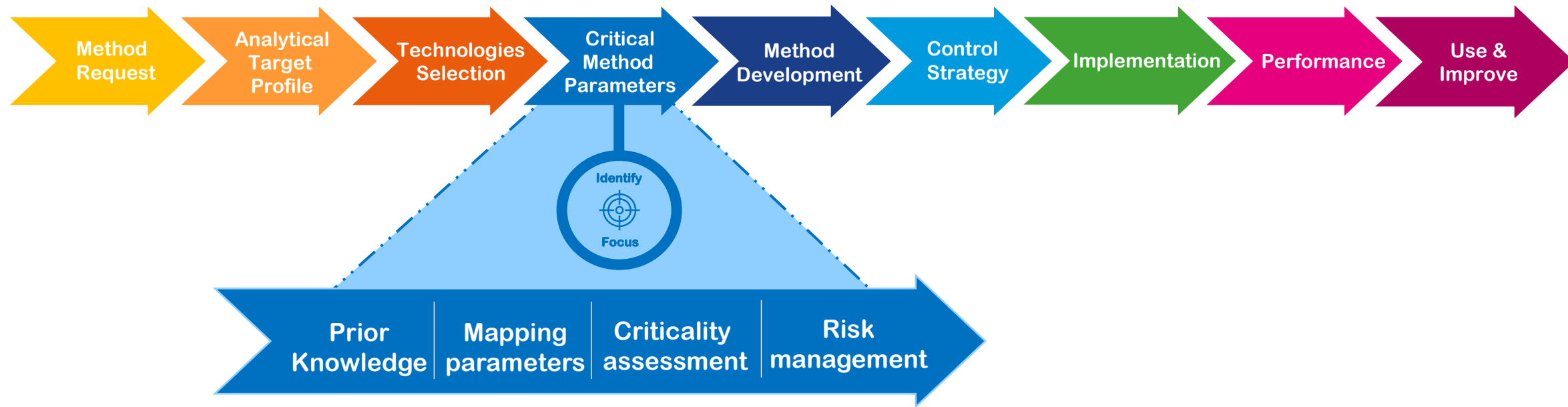
STEP 5: Select 1 or more technologies based on risks and timelines

Capillary Zone Electrophoresis

Capillary Zone Electrophoresis (CZE) is defined as *the movement of charged ions by attraction or repulsion in an electric field*

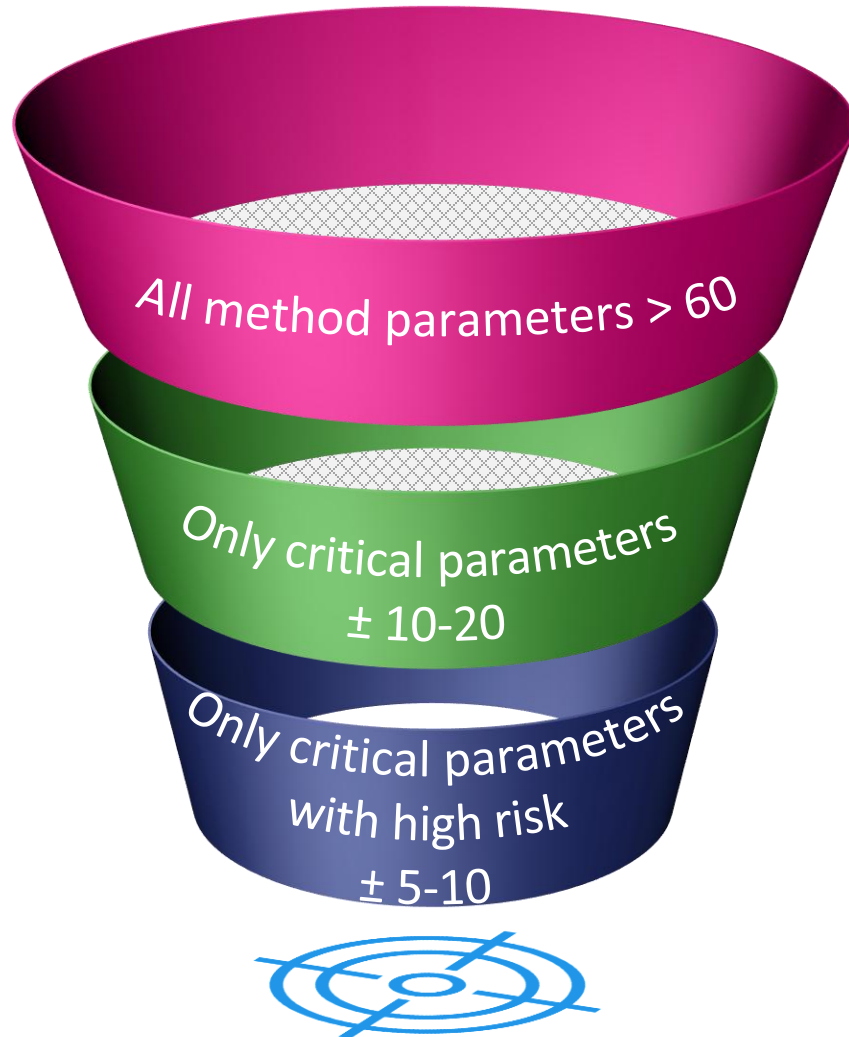


Our AQbD approach - Critical method parameters



CMP = critical method parameter
pCMP = potential critical method parameter
nCMP = non-critical method parameter

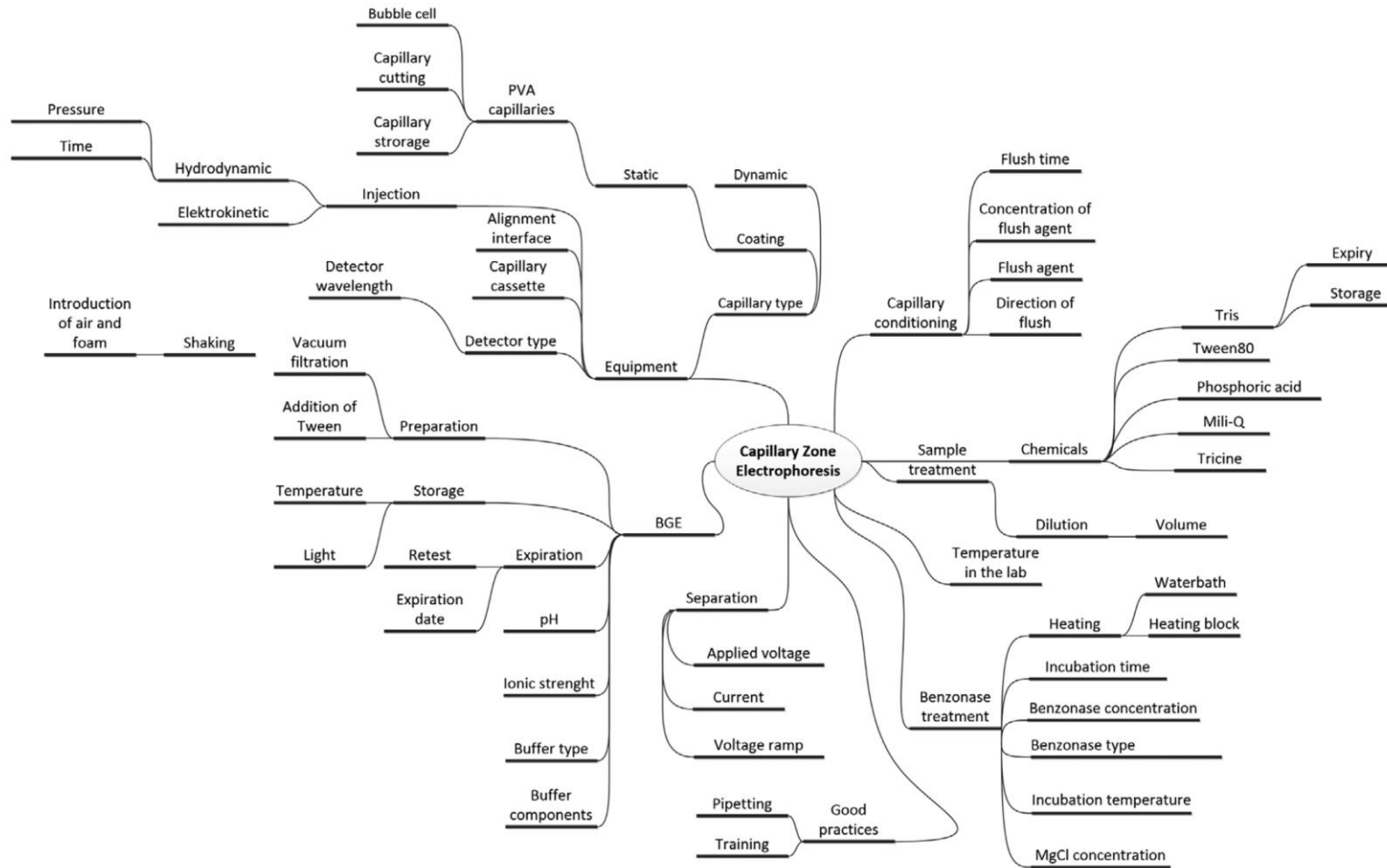
Do we optimize all method parameters?



- Many analytical procedures consist of > 60 parameters that could be optimized and that could possibly impact the ATP requirements
 - Only possible with endless budget, time, and patience

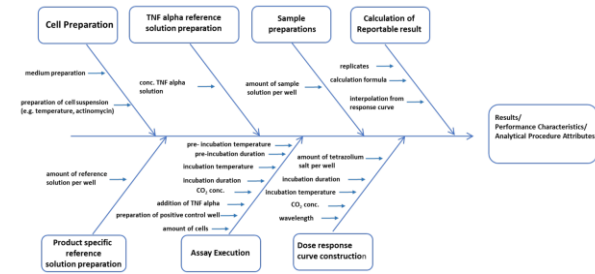
Mapping method parameters

Mind map

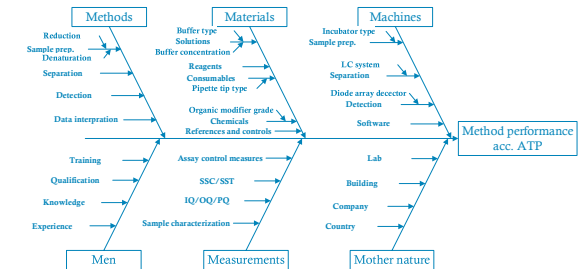


Alternative approaches:

Fishbone - Ishikawa diagram



6Ms (Men, Material, Machine, Mother Nature, Measurement, Method)



Excel list

Method parameters
Buffer pH
Buffer type
Capillary type and coating
Sample type and matrix
Detection wavelength
Injection mode
Capillary storage
Cassette temperature
Gas bubbles in buffer
Particles in buffer
Injection volume
Applied voltage
Sample tray temperature
Sample treatment to remove DNA
Benzonase storage
CE vials
CE lamp equilibration time
Pipette step for sample transfer
Capillary length
Capillary diameter
Capillary alignment interface
Buffer concentration
Benzonase type

Criticality assessment



Method parameters	Effect / Impact (Low, Medium, Significant)	Knowledge / Certainty (Low, Medium, High)	Proceed to risk assessment?	Score
Buffer pH	Significant	high	Yes	CMP
Buffer type	Significant	high	Yes	CMP
Capillary coating	Significant	high	Yes	CMP
Capillary conditioning	Significant	High	Yes	CMP
Sample type and matrix	Significant	high	Yes	CMP
Detection wavelength	Significant	high	Yes	CMP
Injection mode	Significant	high	Yes	CMP
Capillary storage	Significant	high	Yes	CMP
Cassette temperature	Significant	high	Yes	CMP
BGE degassing	Significant	medium	Yes	CMP
BGE filtration	Significant	medium	Yes	CMP
Injection volume	medium	high	Yes	CMP
Applied voltage	medium	high	Yes	CMP
Sample tray temperature	medium	high	Yes	CMP
Sample treatment (remove DNA)	Significant	low	Maybe	pCMP
Benzonase storage	Significant	low	Maybe	pCMP
CE vials	Low	high	No	nCMP
CE lamp equilibration time	Low	high	No	nCMP
Pipette step for sample transfer	Low	high	No	nCMP
Capillary length	Low	high	No	nCMP
Capillary diameter	Low	high	No	nCMP
... (50 additional rows)	...	...	...	...

Scoring table

		Knowledge / Certainty		
		Low	Medium	High
Effect / Impact	Low	pCMP	nCMP	nCMP
	Medium	pCMP	pCMP	CMP
	Significant	CMP	CMP	CMP

Result

- 14 Critical parameters
- 2 Potential critical parameters
- 50+ Non-critical parameters

van Tricht & Sanger-van de Griend,
Electrophoresis, 2025; 46:933–953

CMP = critical method parameter
pCMP = potential critical method parameter
nCMP = non-critical method parameter



1
Mapping all
parameters

2
Score effect (impact) and
knowledge (certainty)

3
List of CMP, nCMP
and pCMP

Criticality
assessment

Method parameter
Buffer pH
Buffer type
Capillary coating
Capillary conditioning
Sample type and matrix
Detection wavelength
Injection mode
Capillary storage
Cassette temperature
BGE degassing
BGE filtration
Injection volume
Applied voltage
Sample tray temperature
Sample treatment (rerun DNA)
Benzonase storage

A paper-based tool to
quickly filter out the non-
critical method parameters

Clear focus achieved by
reducing 60+ method
parameters
to 16 parameters



Knowledge / Certainty		
Low	Medium	High
pCMP	nCMP	nCMP
pCMP	pCMP	CMP
CMP	CMP	CMP

CE vials	Low	high	No	nCMP
CE lamp equilibration time	Low	high	No	nCMP
Pipette step for sample transfer	Low	high	No	nCMP
Capillary length	Low	high	No	nCMP
Capillary diameter	Low	high	No	nCMP
... (50 additional rows)	...	...	...	...

parameters
critical parameters
critical parameters

Tricht & Sanger-van de Griend,
Electrophoresis, 2025; 46:933–953

CMP = critical method parameter
pCMP = potential critical method parameter
nCMP = non-critical method parameter

Risk management

CMP	ATP requirement affected	Possible cause and effect (impact)	Risk score <u>before</u> mitigation			Proposed experiments
			Effect (1-10)	Probability (chance) (1-10)	Score (before mitigation)	
Capillary coating	Bias and precision	Virus adsorption to the capillary wall could impact bias and precision	10	8	80	Test multiple capillaries and coatings and select capillary + coating with good virus recovery
Separation Buffer pH	Bias	A pH that is too low or too high could cause virus degradation	10	8	80	Find the optimal pH range for adenovirus stability
BGE composition	Bias and precision	Inadequate BGE composition could cause the matrix components or adenovirus to adsorb, precipitate, or aggregate and cause an inaccurate and imprecise results	10	8	80	Screening of BGE and capillaries Screening to reduce adsorption Optimize conditions
Sample type and matrix	Bias and precision	Sample matrix components like DNA, protein, or salts could impact the precision and bias	10	8	80	Evaluate whether a sample treatment is required for a set of representative samples from DSP and USP
Detection wavelength	LOD and LOQ	If the wrong detection wavelength is selected, this could result in low or no S/N values.	10	8	80	Evaluate and select a wavelength that allows for adenovirus detection and quantification
...	...	...	...	...	...	...

1

Cause and Effect (impact)

2

Quantify the risk

3a

Proposed Experiments

			Risk score <u>before</u> mitigation			Proposed experiments
CMP	ATP requirement affected	Possible cause and effect (impact)	Effect (1-10)	Probability (chance) (1-10)	Score (before mitigation)	
Capillary coating	Bias and precision	Virus adsorption to the capillary wall could impact bias and precision	10	8	80	Test multiple capillaries and coatings and select capillary + coating with good virus recovery
Separation Buffer pH	Bias	A pH that is too low or too high could cause virus degradation	10	8	80	Find the optimal pH range for adenovirus stability
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...	...	...	...	...	...	...

Risk management

			Risk score before mitigation			Proposed experiments
CM						
Cap coa						Test multiple capillaries and coatings and select capillary + coating with good virus recovery
Sep Buf						Find the optimal pH range for adenovirus stability
BGE con						Screening of BGE and capillaries Screening to reduce adsorption Optimize conditions
San and						Evaluate whether a sample treatment is required for a set of representative samples from DSP and USP
Detection wavelength	LOD and LOQ	If the wrong detection wavelength is selected, this could result in low or no S/N values.	10	8	80	Evaluate and select a wavelength that allows for adenovirus detection and quantification
...	...	...	...	...	...	...

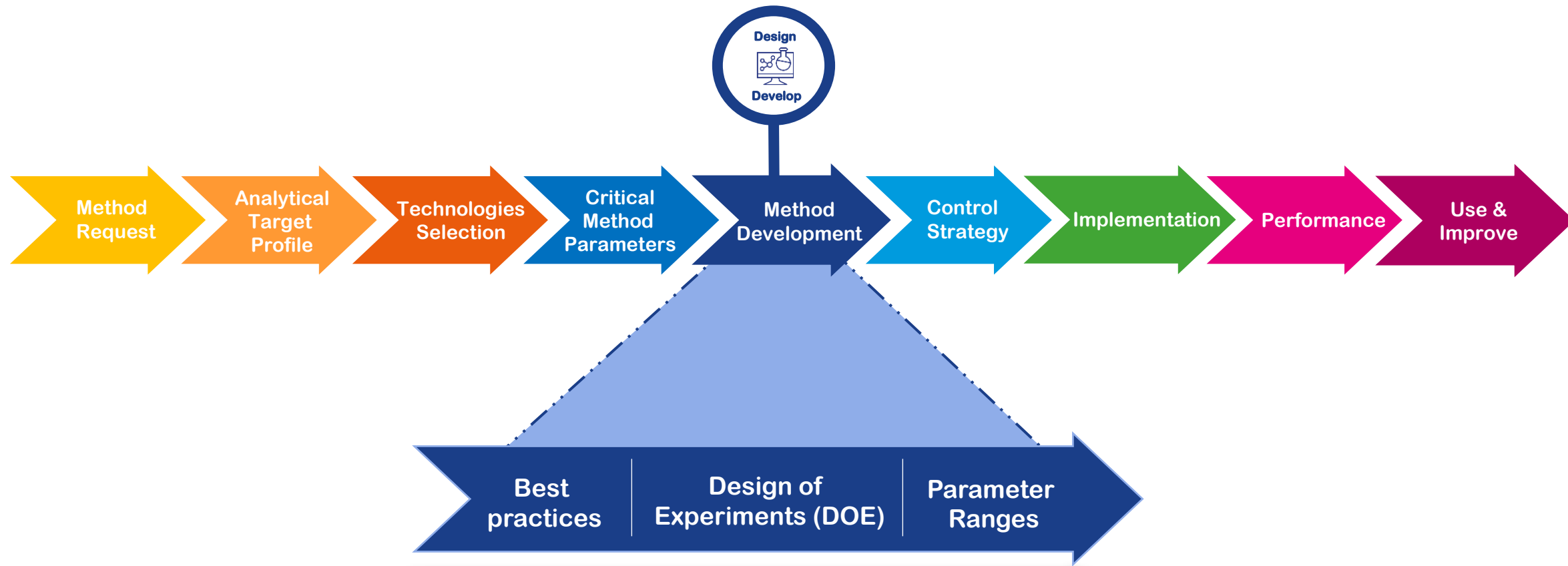


Critical method parameters with highest risk:

- Capillary type
- Capillary coating
- Buffer type
- Buffer pH

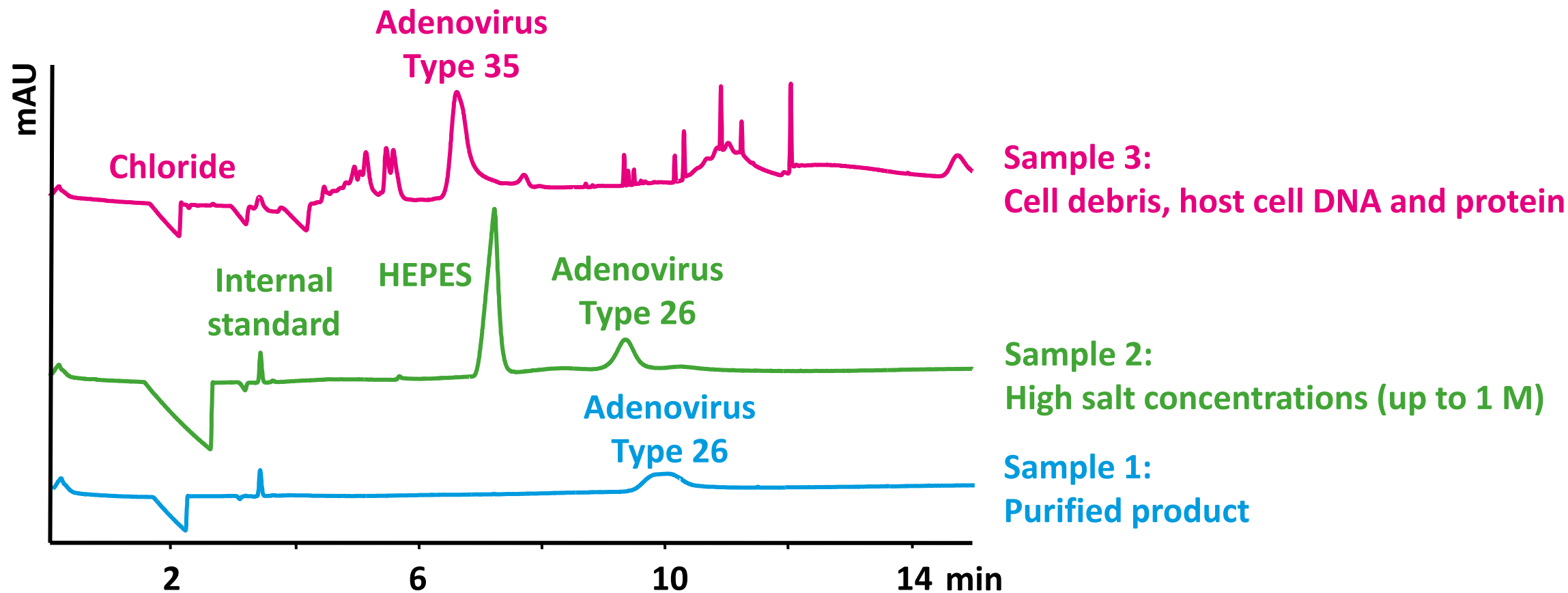
Focus of method development

Our AQbD approach - Method development



Method development – initial results

Capillary electrophoresis results

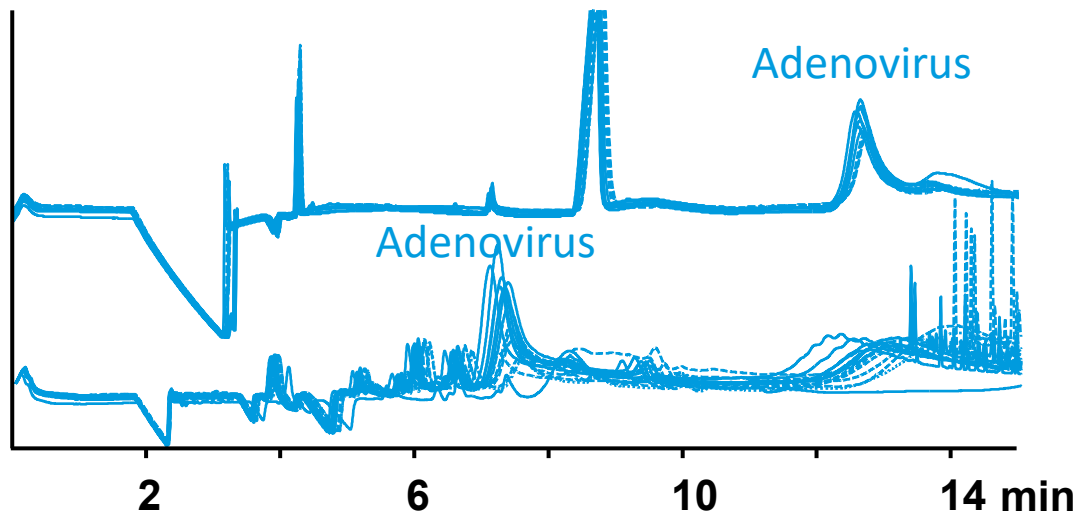


Conditions: BGE tris 200 mM - 200mM tricine, pH 8.1; -15kV; neutral coated capillary 33 cm, 50 μ m ID; injection: 50 mbar x 5 s; detection: 214 nm

Development - adsorption

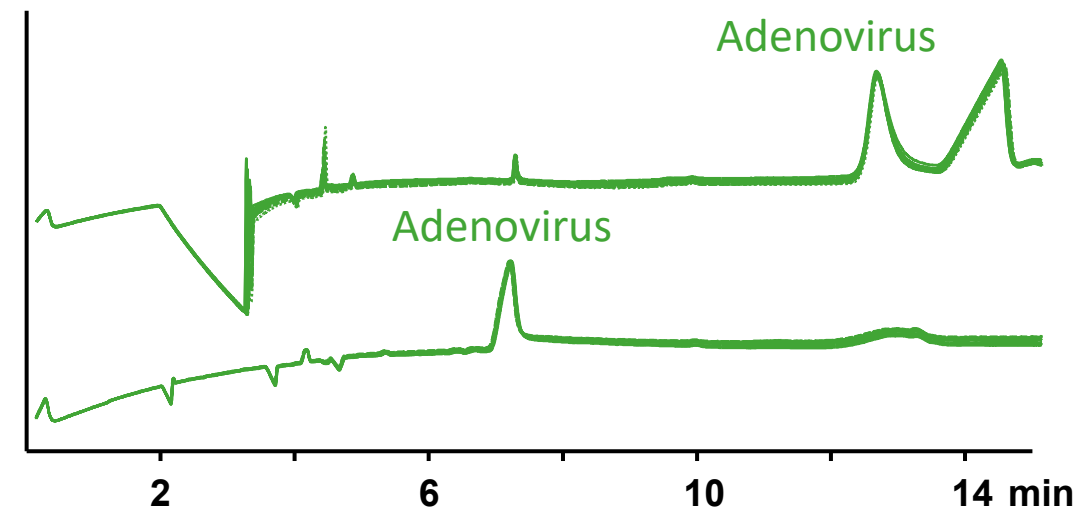
The following conditions did not prevent or reverse adsorption:

- BGE: 100:100 mM tris:tricine, pH 8.3
- Cassette temperature: 25 °C
- Flushing agent: BGE, milli-Q, SDS, or 2-mercaptoethanol
- Additives: triton or SDS



The following conditions did prevent or reverse adsorption:

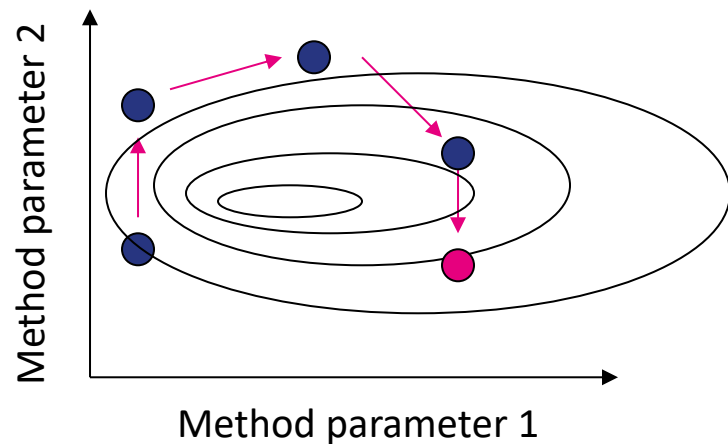
- BGE: 125:375 mM tris:tricine, pH 7.6
- Cassette temperature: 15 °C
- Flushing agent: 2 min flush with 10 mM phosphoric acid
- Additive: Tween20: 0.2% v/v



Design of experiments (DoE)

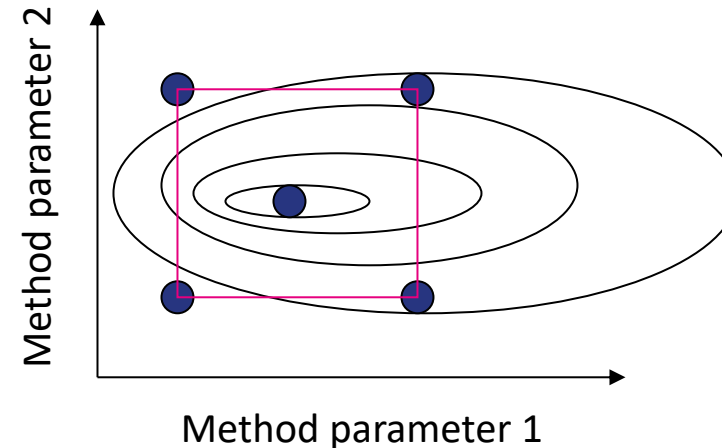
OFAT (one factor at a time)

- Trial and error/success
- Change one thing at the time
 - Better or worse?
 - Change a bit more
- Interactions between method parameters cannot be estimated
- Optimal settings can only be found with luck



DoE Multivariate approach

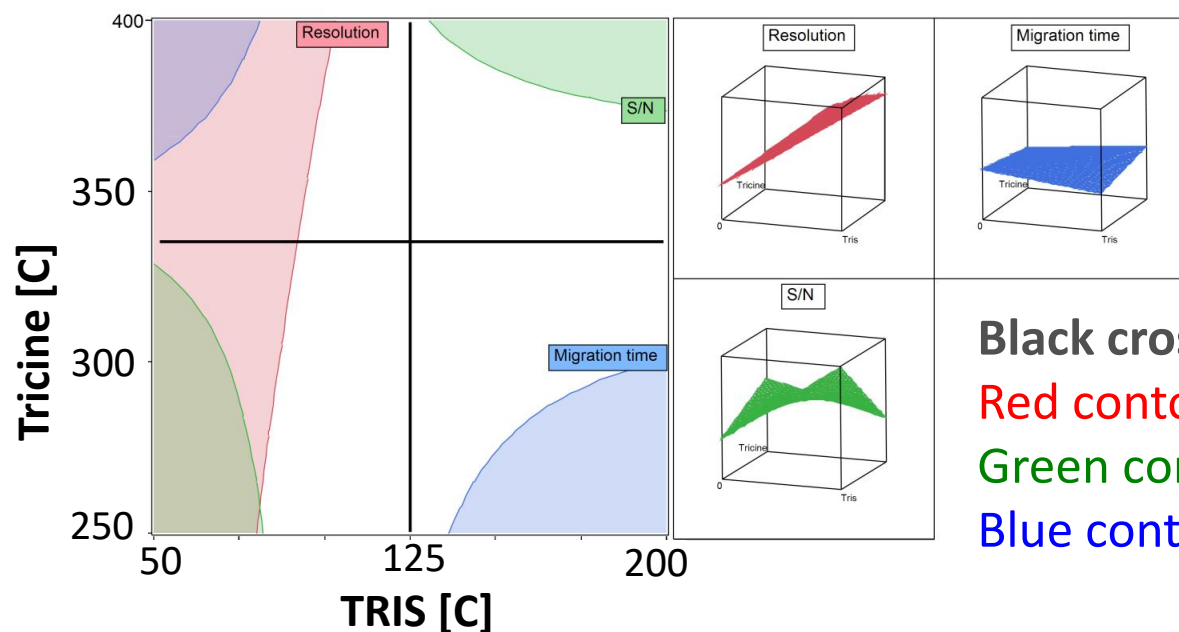
- Structured
- Systematic
- Planned
- Interaction of method parameters can be identified and quantified
- Find the real optimal settings and information of design space



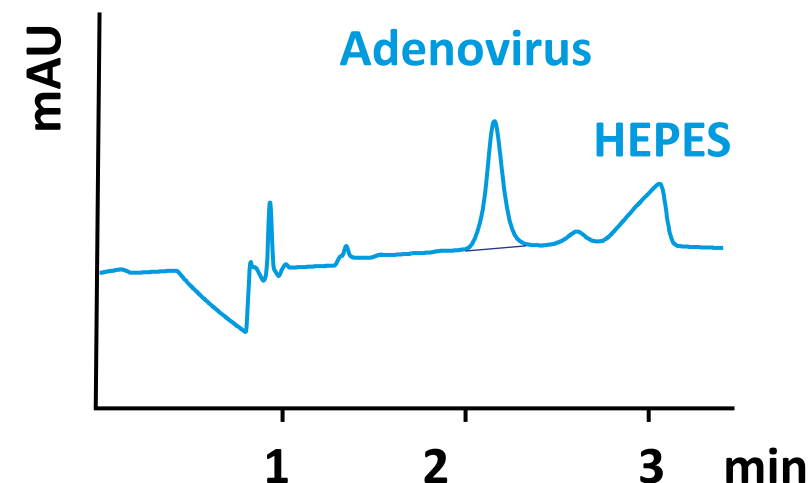
Optimization DOE

Method parameters tested:

- Voltage: -15, -20, -25, -30 kV
- Tris: 50, 125, 200 mM
- Tricine: 250, 325, 400 mM
- Short-end and Long-end of capillary (24.5 cm and 8.5 cm effective length)



Electropherogram at selected optimum conditions:



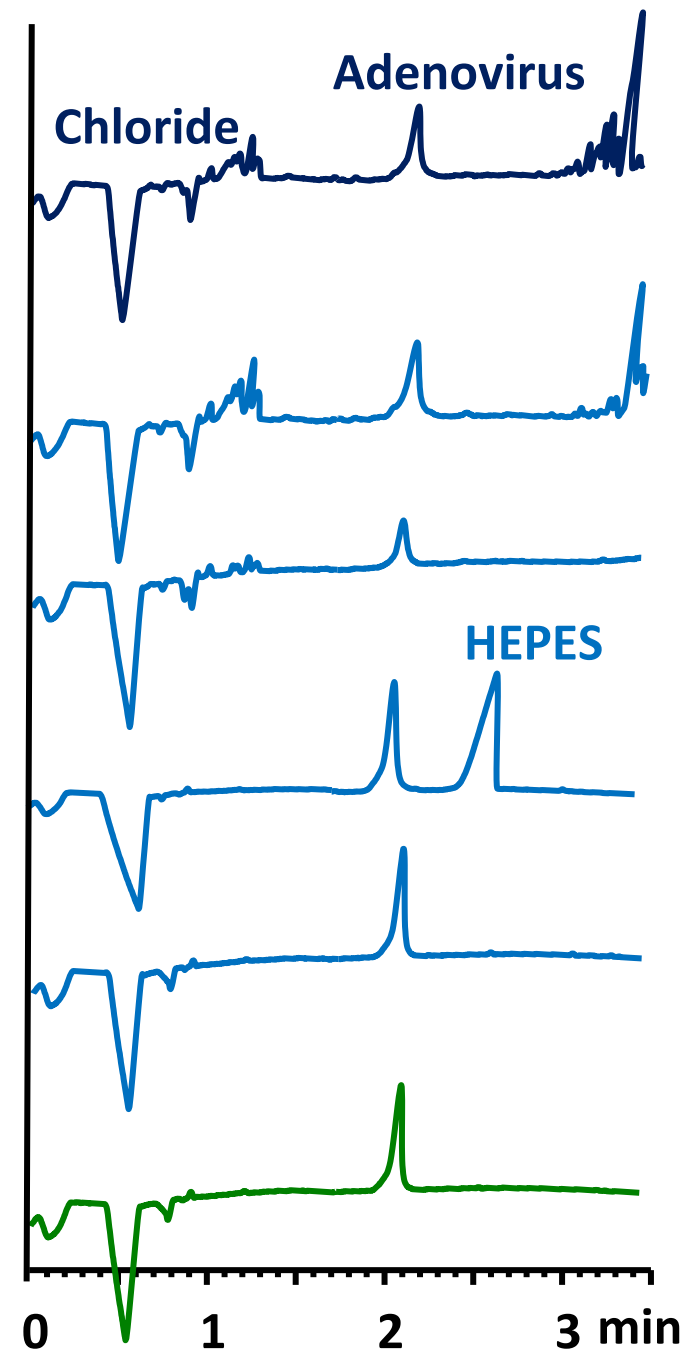
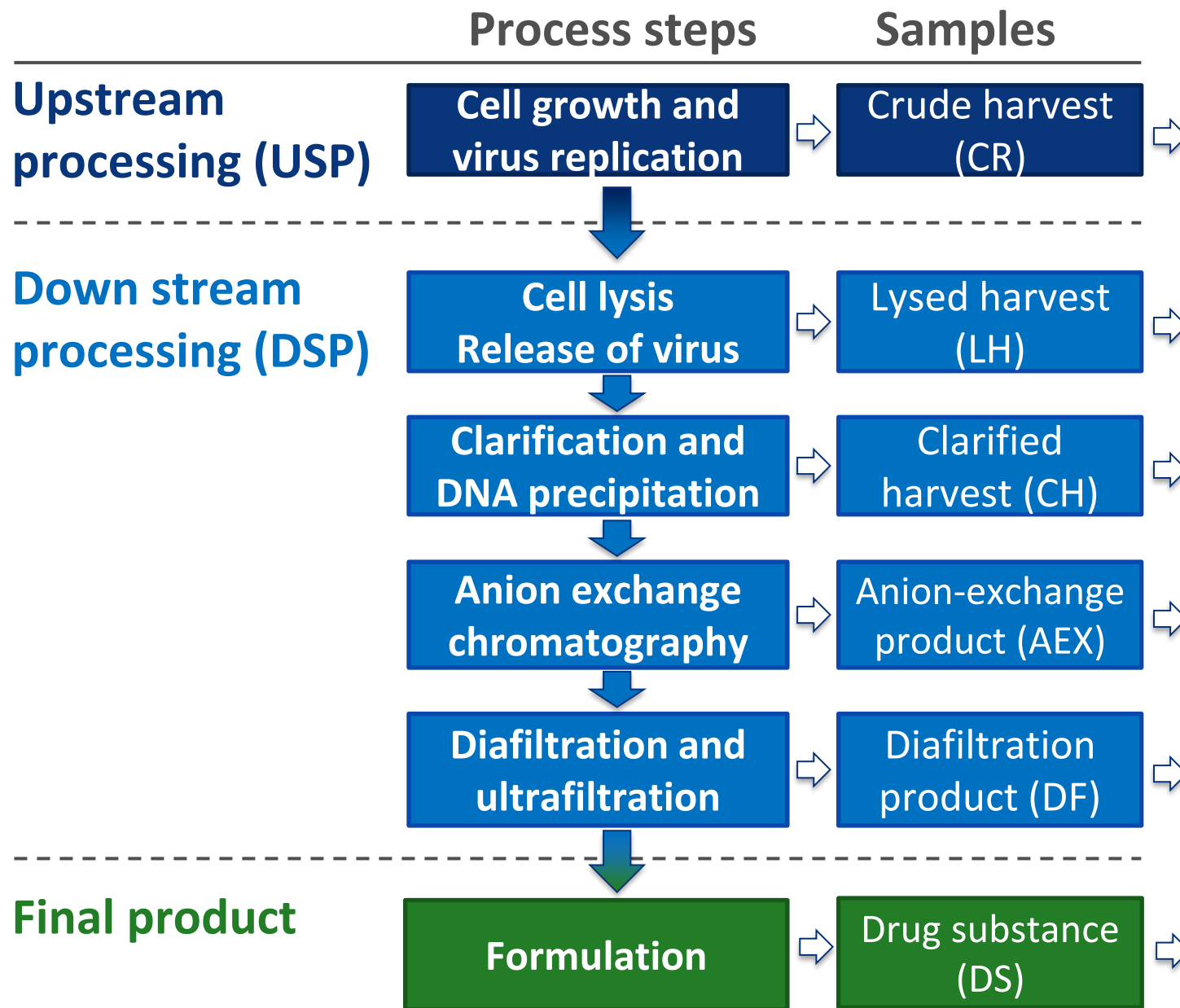
Black cross marker: Selected conditions

Red contour: Min. resolution of 2.5

Green contour: Min. Signal-to-noise ratio of 150

Blue contour: Max. run time of 6 min

Method development – final results

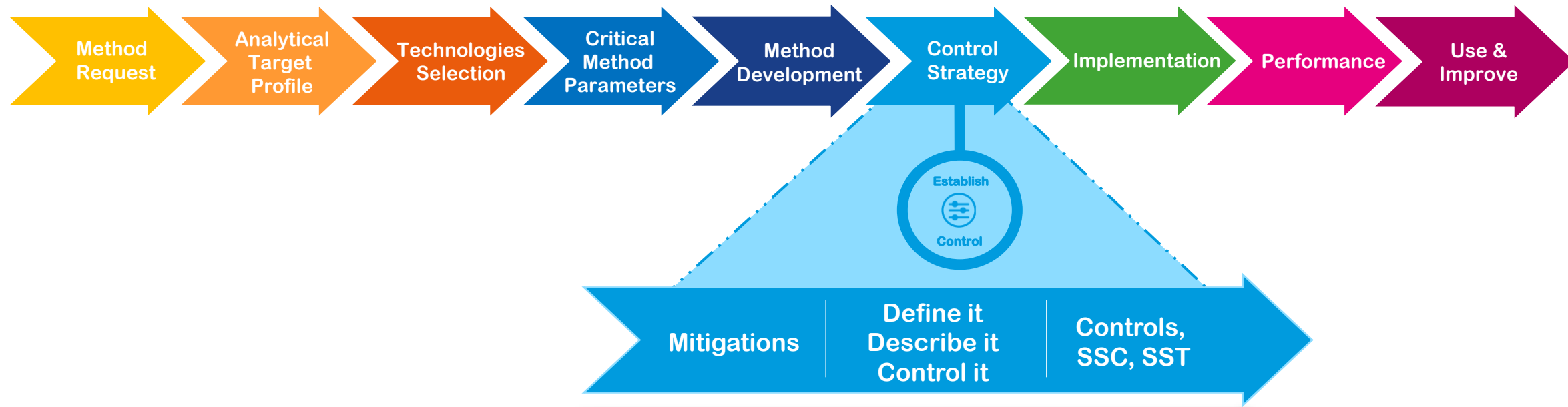


We are done! Method is developed!

As CE was a new technology, implementation and life-cycle management took 30 – 40% of the total time



Our AQbD approach - Control strategy



How to get into control over each method parameter?

Three ways to mitigate and reduce the risk for your critical method parameters:

Define it

Set a fixed value or range for a parameter

Set the buffer pH to 7.6 ± 0.1 to prevent virus degradation.

Describe it

Provide a step-by-step procedure for executing a parameter

Mix the sample by pipetting up and down 5 times with a 100 μ l pipette.

Control it

Implement a verification mechanism to monitor a parameter

Use an adenovirus control sample at $1 \cdot 10^{11}$ VP/mL to verify accuracy (85 – 115%).

This control strategy is based on **experiments, theory, literature, and common knowledge.**

VP/mL = virus particles per milliliter

Risk management

1

Cause and Effect (impact)

2

Quantify the risk

3a

Proposed Experiments

			Risk score <u>before</u> mitigation			Proposed experiments
CMP	ATP requirement affected	Possible cause and effect (impact)	Effect (1-10)	Probability (chance) (1-10)	Score (before mitigation)	
Capillary coating	Bias and precision	Virus adsorption to the capillary wall could impact bias and precision	10	8	80	Test multiple capillaries and coatings and select capillary + coating with good virus recovery
Separation Buffer pH	Bias	A pH that is too low or too high could cause virus degradation	10	8	80	Find the optimal pH range for adenovirus stability
BGE composition	Bias and precision	Inadequate BGE composition could cause the matrix components or adenovirus to adsorb, precipitate, or aggregate and cause an inaccurate and imprecise results	10	8	80	Screening of BGE and capillaries Screening to reduce adsorption Optimize conditions
Sample type and matrix	Bias and precision	Sample matrix components like DNA, protein, or salts could impact the precision and bias	10	8	80	Evaluate whether a sample treatment is required for a set of representative samples from DSP and USP
Detection wavelength	LOD and LOQ	If the wrong detection wavelength is selected, this could result in low or no S/N values.	10	8	80	Evaluate and select a wavelength that allows for adenovirus detection and quantification
...	...	...	...	...	...	...

Risk management

1

Cause and Effect (impact)

2

Quantify the risk

3a

Proposed
Experiments

3b

Mitigations
(define, describe,
control)

4

Reassess the risk

			Risk score <u>before</u> mitigation					Risk score <u>after</u> mitigation		
CMP	ATP requirement affected	Possible cause and effect (impact)	Effect (1-10)	Probability (chance) (1-10)	Score (before mitigation)	Proposed experiments	Mitigations (define it, describe it, control it)	Effect (1-10)	Probability (chance) (1-10)	Score (after mitigation)
Capillary coating	Bias and precision	Virus adsorption to the capillary wall could impact bias and precision	10	8	80	Test multiple capillaries and coatings and select capillary + coating with good virus recovery	A PVA coated capillary was selected to prevent adsorption of the adenovirus. Optimized capillary conditioning in between injections.	10	3	30
Separation Buffer pH	Bias	A pH that is too low or too high could cause virus degradation	10	8	80	Find the optimal pH range for adenovirus stability	The optimal pH range was determined as pH 6.0 – 8.5.	10	1	10
BGE composition	Bias and precision	Inadequate BGE composition could cause the matrix components or adenovirus to adsorb, precipitate, or aggregate and cause an inaccurate and imprecise results	10	8	80	Screening of BGE and capillaries Screening to reduce adsorption Optimize conditions	Use tris/tricine buffer. Add PS-20 to BGE. Set BGE to 125 mM tris and 338 mM tricine and 0.2% w/v PS-20. Introduce an adenovirus control sample to monitor accuracy and precision	10	1	10
Sample type and matrix	Bias and precision	Sample matrix components like DNA, protein, or salts could impact the precision and bias	10	8	80	Evaluate whether a sample treatment is required for a set of representative samples from DSP and USP	A sample treatment was implemented for USP samples to remove residual DNA. The precision and bias were determined and acceptable for three representative samples: CH, AEX, and DS	10	3	30
Detection wavelength	LOD and LOQ	If the wrong detection wavelength is selected, this could result in low or no S/N values.	10	8	80	Evaluate and select a wavelength that allows for adenovirus detection and quantification	A wavelength of 214 nm was selected to analyze adenovirus.	10	1	10
...	...	...	...	...	...	...	...	...	...	...

Risk management

All critical method parameters are known and in control



All the knowledge is captured and traceable and shareable

Probability (chance) (1-10)	Score (after mitigation)
3	30
1	10
1	10
3	30
1	10
...	...

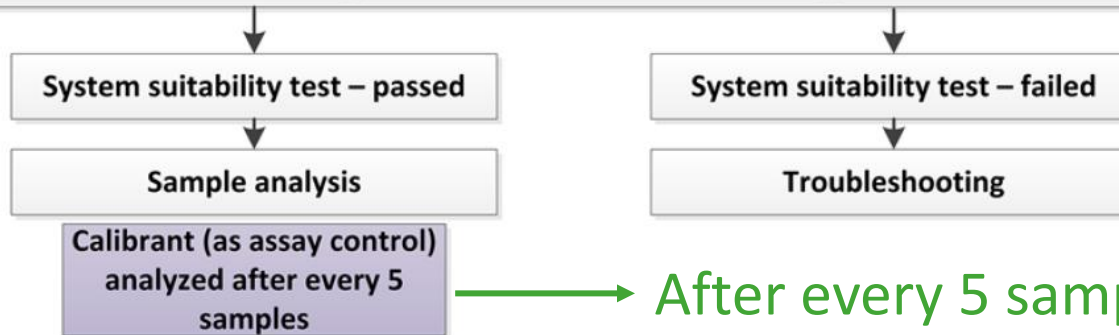
System suitability testing based on ATP requirements

A very stringent SST - checking all the critical aspects of the analytical method

System suitability test		
Test	Rationale	Criteria
Fill capillary with BGE and apply voltage	Check the BGE preparation and capillary quality (cut, blockage)	Current: 40 – 60 μA
2 Injections: blank	- Test injection - Check for system contamination	- Negative triangular chloride peak between 0.4–0.7 min - Signal < 0.45 mAU between 1.0–3.5 min
6 injections: adenovirus SSC at 1×10^{11} vp/ml	- Confirm precision and bias - Check for capillary coating quality (absence of adenovirus adsorption)	- Adenovirus peak (with a UV spectrum match >950) between 1.8–2.5 min - Adenovirus concentration: $0.85\text{--}1.15 \times 10^{11}$ vp/ml - Migration time repeatability: <1%RSD - Peak area repeatability: <5%RSD
1 injection: blank	Check for carry-over	- Negative triangular chloride peak between 0.4–0.7 min - Signal < 0.45 mAU between 1.0 and 3.5 min
1 injection: Calibrant at 7.3×10^{11} vp/ml	Used to determine the concentration of the SSC and samples (if the SST passed)	Adenovirus peak (with a UV spectrum match >950) between 1.8–2.5 min

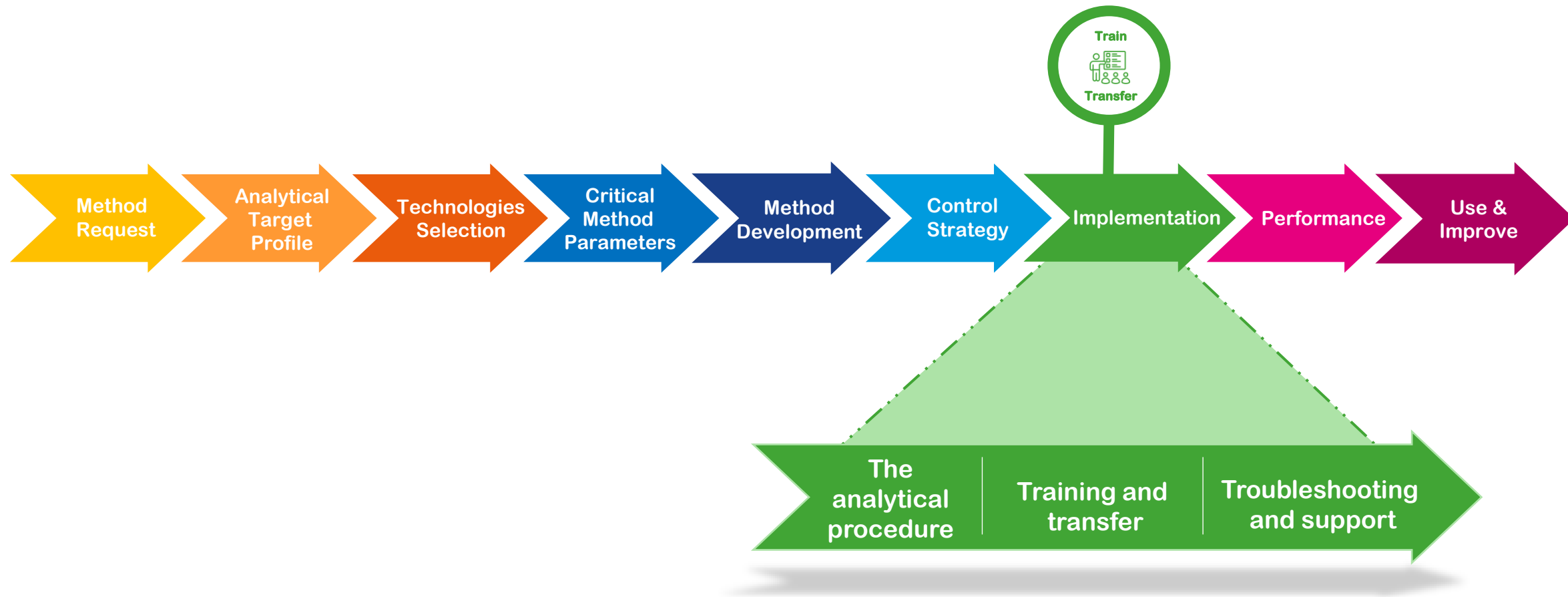
Check if the capillary is properly cut, undamaged, and intact.

The adenovirus concentration should be $1 \cdot 10^{11}$ VP/mL $\pm$ 15% (based on expected total error)



After every 5 sample injections, the total error of the method is confirmed

Our AQbD approach - Implementation



Method implementation

The analytical procedure

Write the analytical procedure as precisely as possible, covering all critical details

Standard Operating Procedure

Capillary Electrophoresis Method for Direct Quantification of Intact Adenovirus Particles

Version: 1.0

Document number : CAL-CE-005

Page 1 of 7

PURPOSE

This procedure describes the CE method for rapid, robust and accurate determination of intact adenovirus particles in USP and DSP samples.

Focus on:

Define it

Describe it

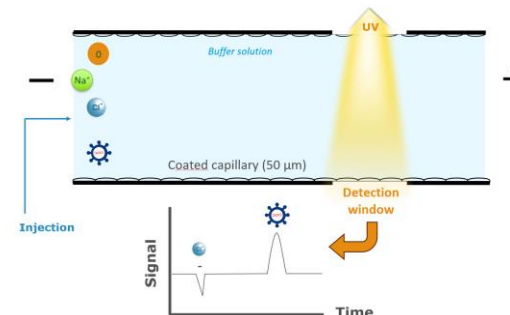
Control it

Knowledge transfer and training

Risk assessment contains all critical knowledge

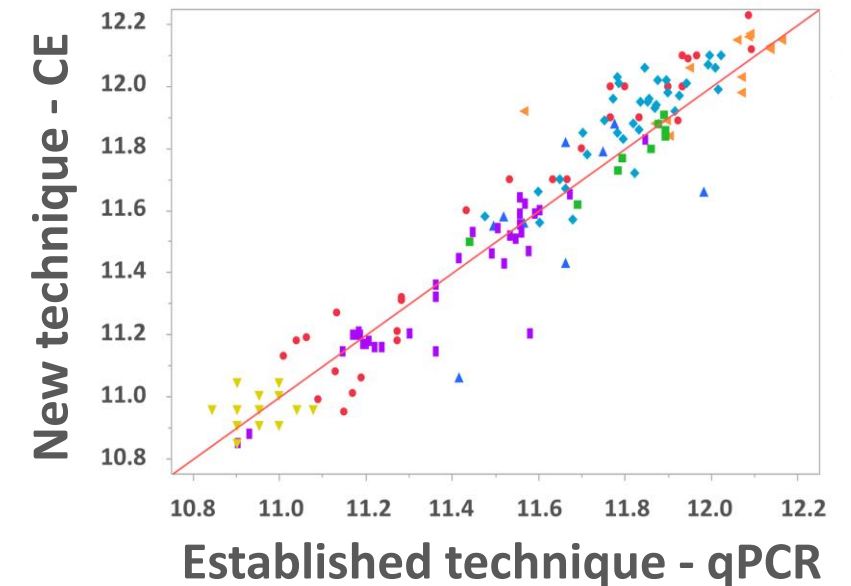
USP	ATP requirement reference	Possible cause and effect (impact)	Effect (1-10)	Probability (low-high)	Score (before mitigation)	Proposed experiments	Mitigation (define & describe it, control it)	Effect (1-10)	Probability (low-high)	Score (after mitigation)
Capillary coating	Run and precision	Virus adsorption to the capillary wall could impact bias and precision	10	8	80	Test multiple capillaries and coatings and select capillary + coating with good virus recovery	A PVA coated capillary was selected to prevent adsorption of the adenovirus. Optimized capillary conditioning is between 10 minutes	10	3	30
Separation buffer pH	Run	A pH that is too low or too high could cause virus degradation	10	8	80	Find the optimal pH range for adenovirus stability	Use Tris-HCl buffer. Set pH to 8.0-8.5. The optimal pH range was determined as pH 8.0-8.5.	10	1	10
BSL compensation	Run and precision	Inadequate BSL compensation could cause the matrix components or substances to affect the precision or accuracy and cause an inaccurate and imprecise result	10	8	80	Screening of BSL and capillaries	Use Tris-HCl buffer. Set pH to 8.0-8.5. Set BSL to 1.0 mV/cm and 100 mV. Set BSL to 0.25 mV/cm. Introduce an adenovirus control sample to monitor accuracy and precision	10	1	10
Sample type and matrix	Run and precision	Systemic conditions (sample matrix components like DNA, protein, or salts) could impact the precision and bias	10	8	80	Systemic conditions (Evaluate whether a sample treatment is required for a set of representative samples from USP and DSP)	A sample treatment was implemented for USP samples to remove residual DNA. The precision and bias were determined and acceptable for three representative samples (1, 4, 5, and 6)	10	3	30
Detection wavelength	LOD and LOQ	If the wrong detection wavelength is selected, this could result in low or no UV values	10	8	80	Evaluate and select a wavelength that allows for adenovirus detection and quantification	A wavelength of 214 nm was selected to analyse adenovirus.	10	1	10

Method specific training + theoretical and technology training



Method comparison

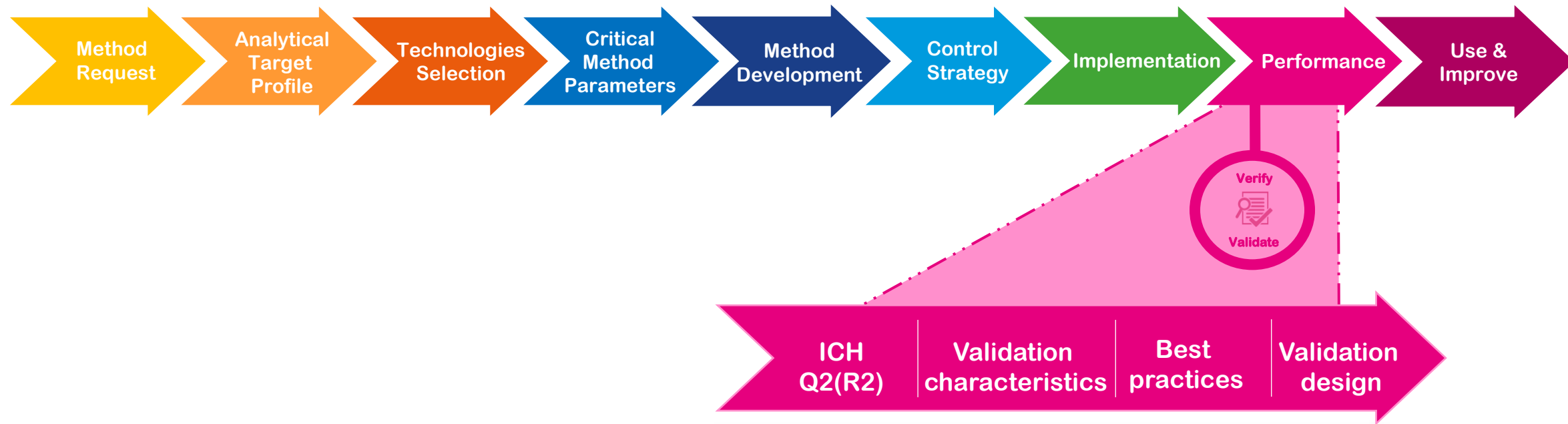
Is the historical data still comparable?



Process intermediates and final product

- 1. Crude harvest
- 2. Lysed harvest
- 3. Clarified harvest
- 4. Anion-exchange product
- 5. Diafiltration product
- 6. Drug substance
- 7. Drug product

Our AQbD approach - Performance



Method validation – total error

Method successfully co-validated
between AD and QC departments

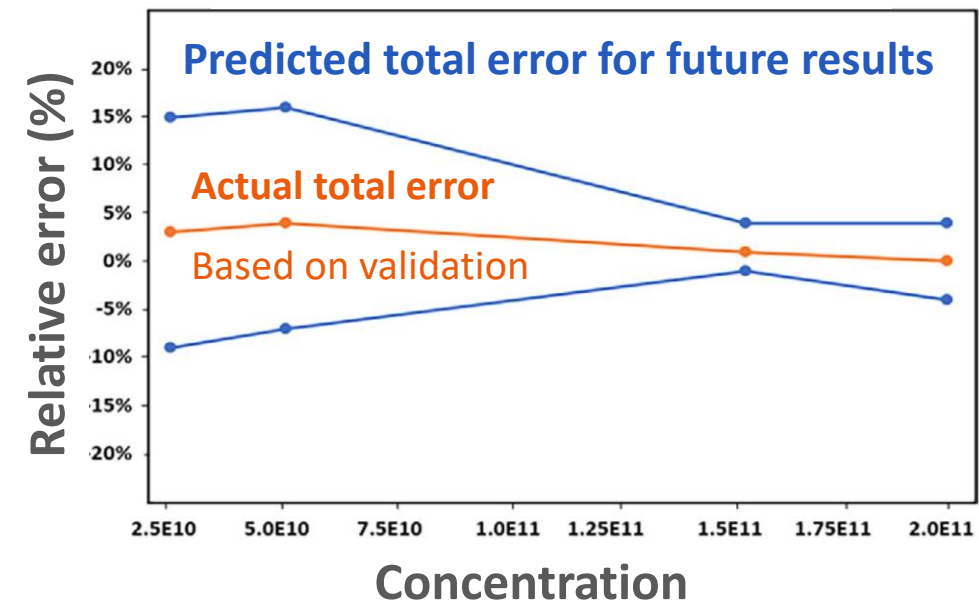
Validation parameter	Acceptance criteria	Result
Method repeatability	< 10% CV	1.1 – 4.3% CV
Intermediate precision	< 10% CV	1.3 – 5.2% CV
Linearity	90% CI of slope between 0.9 and 1.1	0.93 – 0.99
Accuracy (spiked recovery)	90 – 110%	91 – 109%
Range	$0.5 \cdot 10^{11}$ to $1.5 \cdot 10^{11}$ adenovirus particles per ml	Pass

How well was my method performing
in that validation experiment

Total error approach

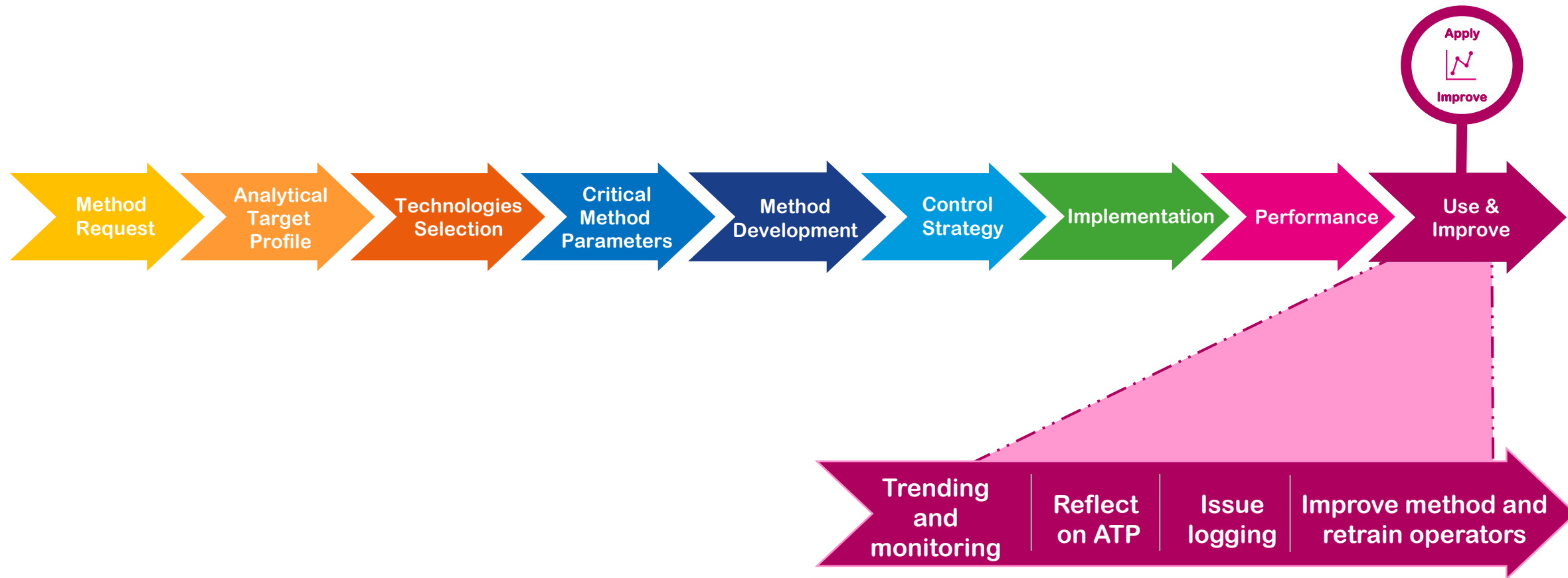
Total error = accuracy error + precision error

Total error profile

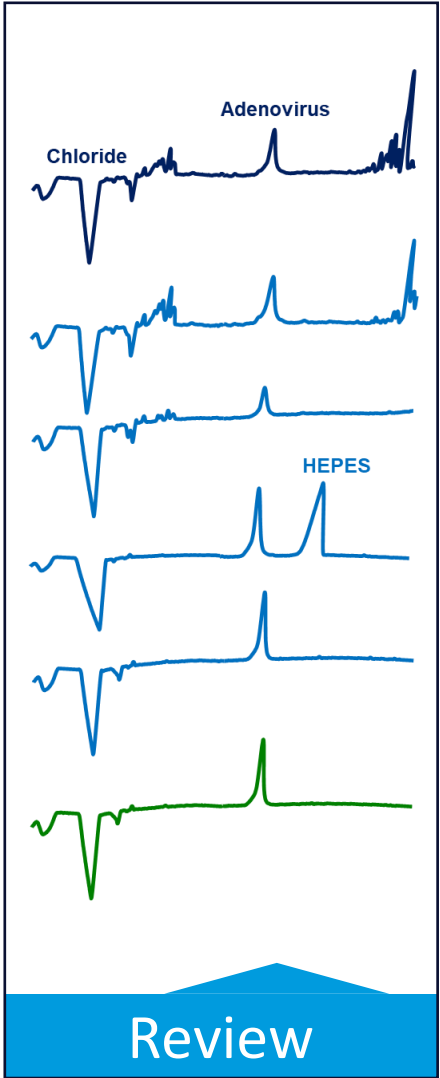


How well will the method perform in the future
A maximum total error of 15%

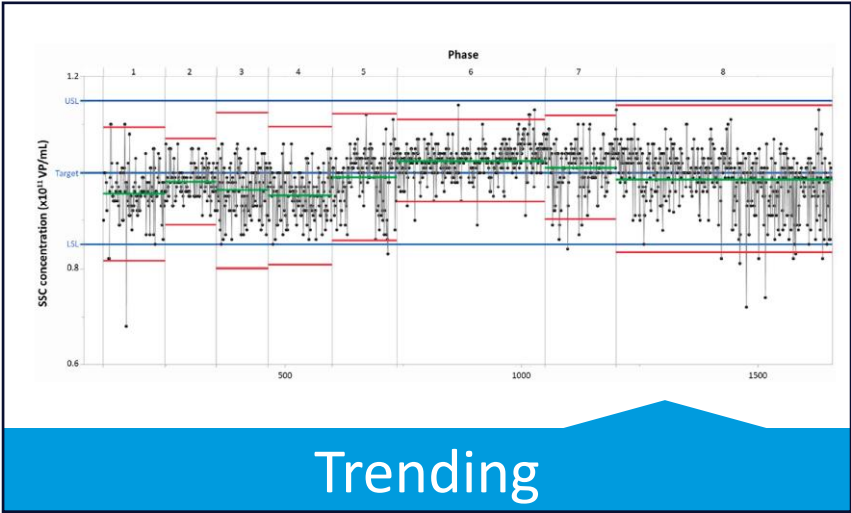
Our AQbD approach - Use & Improve



Use and Improve



Review



Trending

Summary of request		Priority	Targets and ranges	
Product quality attribute			Virus particle concentration determination (in VP/ml)	
Clinical phase			Phase I - commercial	
Aim of study/method			1) For adenovirus production process development and product characterization a precise, accurate, robust, degradation sensitive, and a high-throughput method is needed for the quantification of adenovirus particles in all process intermediates, OS, and DP. 2) An accurate adenovirus amount should be loaded upon the AEX filters, to avoid filter blockage, excessive viral dilution, and impurity break through and to avoid product degradation whilst testing, a precise, accurate, robust, and fast method for adenovirus quantification is required for in-process control testing (IPC).	
Product description and/or process step		1	Crude harvest, Lysed harvest, Clarified harvest (IPC), Anion exchange product, Ultrafiltration product, Drug substance, Drug product	
Analyte(s)		1	Adenovirus type 26 and 35	
Matrices			The matrix could contain cell debris, residual DNA, salt, protein and or formulation buffer components dependent on the process intermediate	
Concentration range(s)		3	10 ¹⁰ ·10 - 10 ¹² VP/ml	
Requester requirements		Time-to-result	< 2 days for process development (1), < 4 h for IPC testing (2)	
Type of test			Content / potency	
Required validation status			Qualified	
ICH Q2-characteristics		Specificity	Detection of Ad26 and Ad35 without significant interference of sample matrix components	
		Range / linearity	1x10 ¹⁰ - 1x10 ¹² VP/ml	
		Accuracy	≤ 10% bias	
		Precision / Intermediate Precision	2	Intermediate precision ≤10% RSD
End-user			Quality control	
End-user requirements		Throughput	3	1 sample per run for IPC testing, > 7 samples per process development run, > 300 samples a month for process development

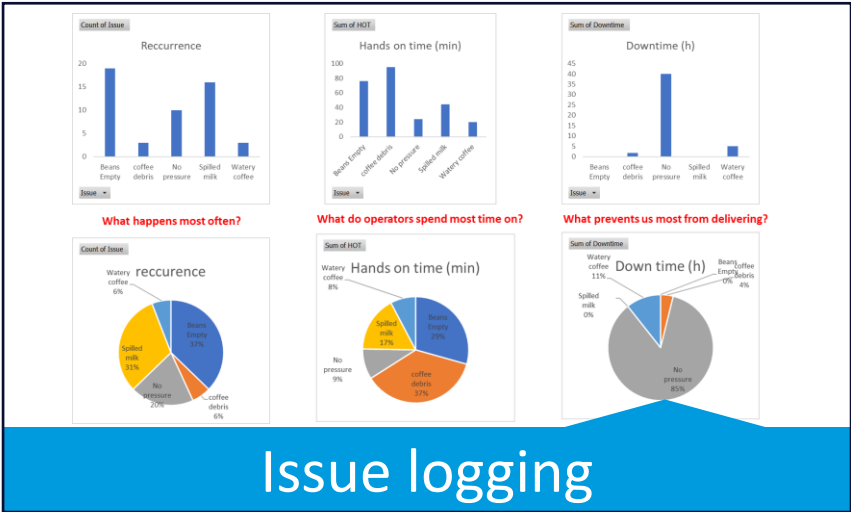
REQUESTOR

METHOD DEVELOPER

END-USERS

Reflect on the ATP

Reflect on the ATP



Issue logging



Method improvement and retraining

Improved technique: Capillary Electrophoresis

Bioreactor used
for production of
vaccines



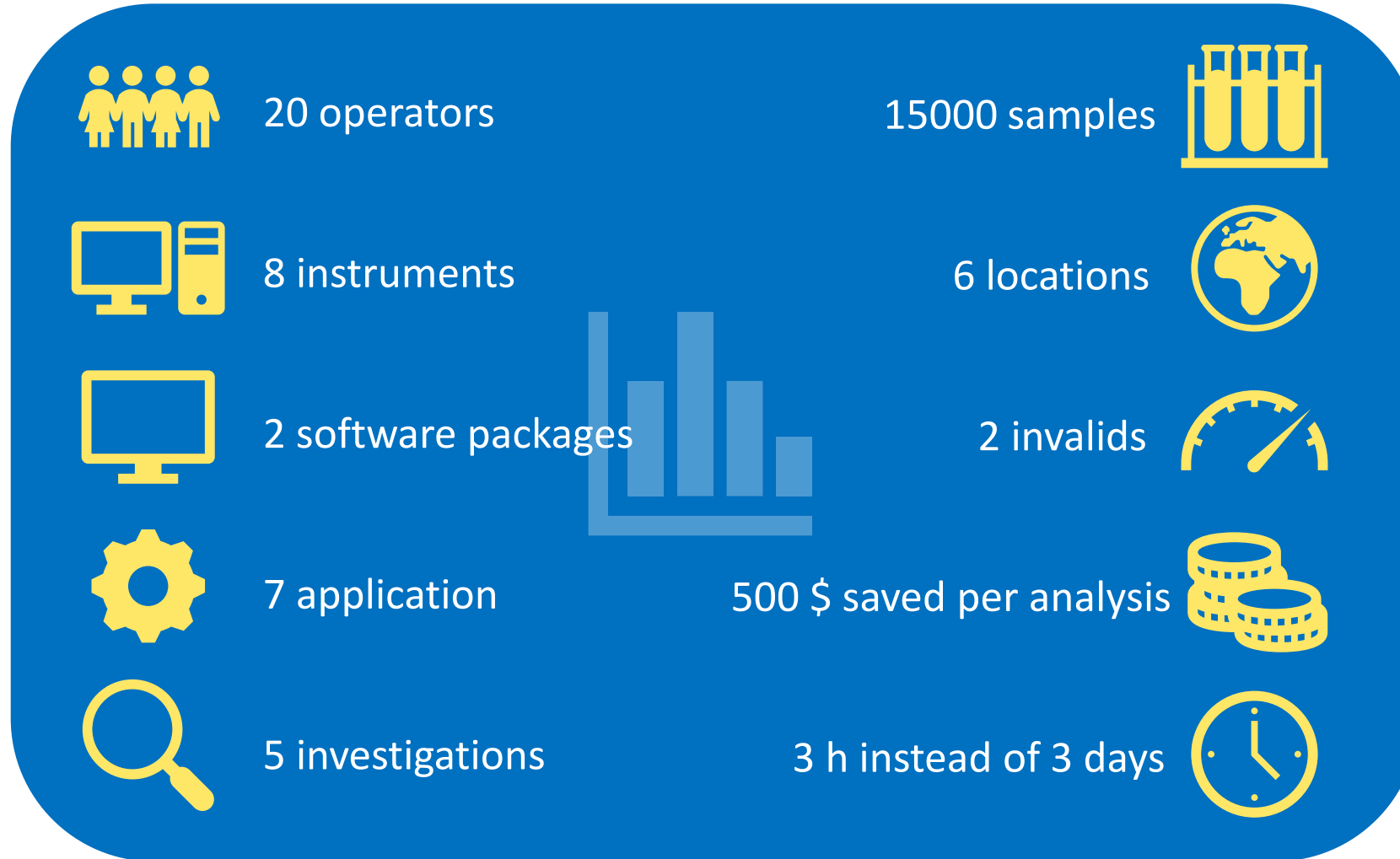
+

Capillary
electrophoresis
instrument



The CE instrument was installed next to the bioreactor.
The **virus particle concentration** could be determined
at any time during the production **within 1 hour**
(previously 3 days)

Statistics (after 3 years)



What does AQbD mean to me?

RIGHT METHOD FOR PURPOSE

- Only develop methods with clear request
- Handshake with requester

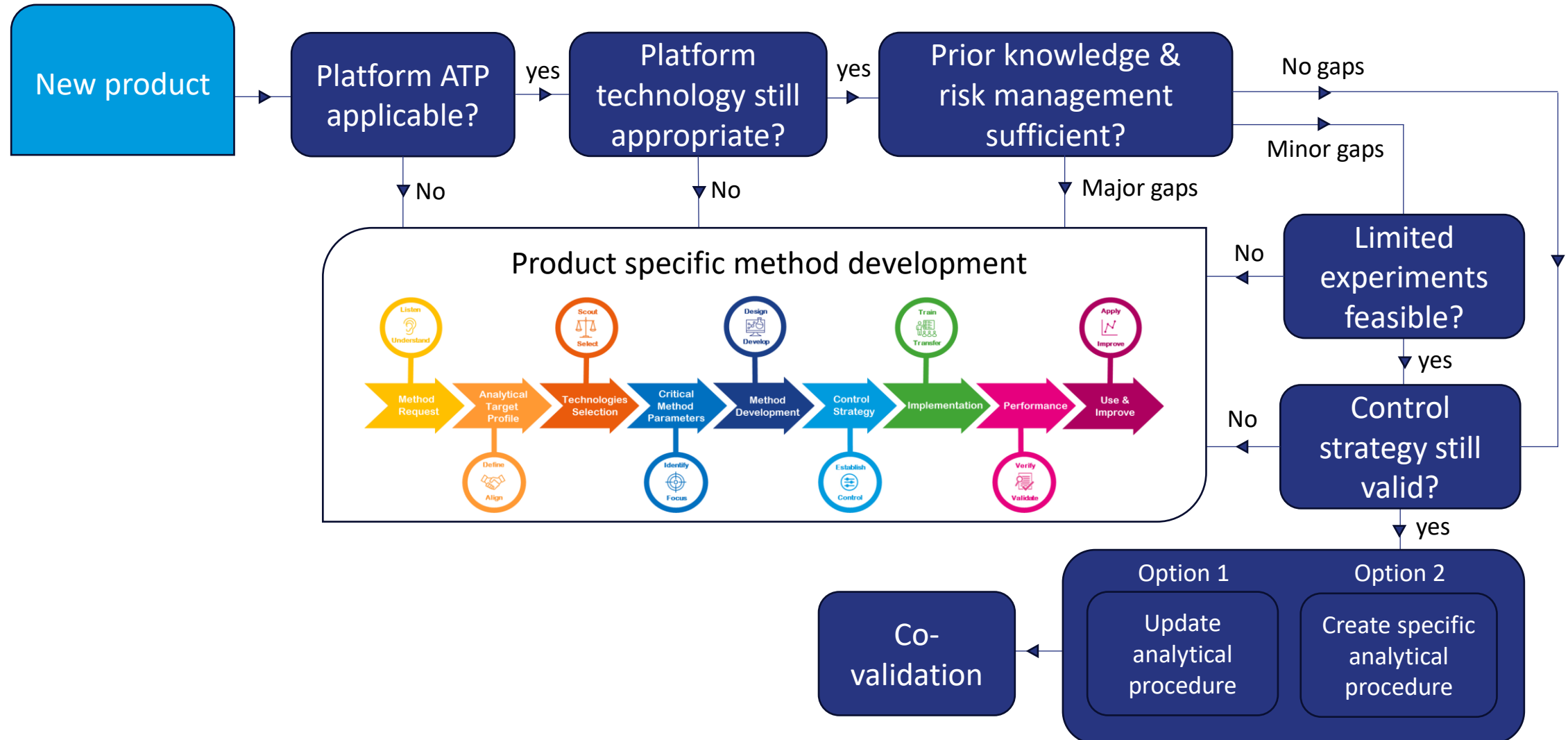
STRUCTURE

- Everyone uses same approach
- Knowledge is captured, re-usable and shareable

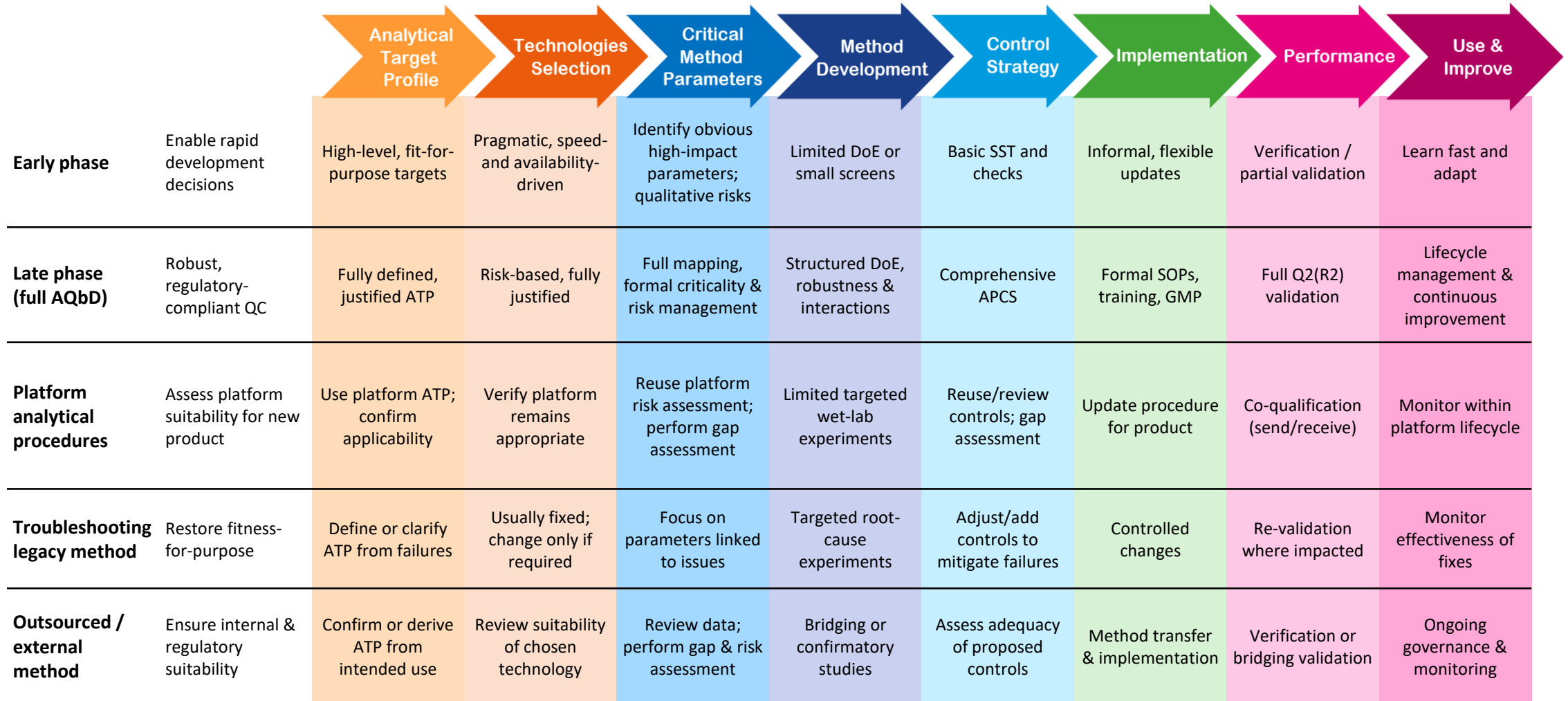
CONTROL

- Sources of variation are understood
- Critical method parameters are known and under control

Our approach for platform development



Our Phase-dependent AQbD-approach



Latest publications by Kantisto

Electrophoresis

WILEY-VCH

ELECTROPHORESIS

RESEARCH ARTICLE **OPEN ACCESS**

Improvement of Peak Integration in Capillary Electrophoresis: Reference Data Set No. 1

Marlon Krompholz¹ | Timothy Blanc² | Huixin Lu³ | Patricia Christensen⁴ | Frédéric Ginot⁵ | Gábor Járvas⁶ | Trang D. Nguyen⁷ | Ashley Prout⁴ | Timothy Riehlman⁸ | Brian Wei⁹ | Andrei Hutanu¹⁰ | Steffen Kiessig¹⁰ | Knut Baumann¹ | Cari E. Sängers-van de Griend^{1,11,12} | Hermann Wätzig¹

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Received: 10 December 2025 | Revised: 14 January 2026 | Accepted: 19 January 2026

<https://doi.org/10.1002/elps.70077>

Latest publications by Kantisto

Analytica Chimica Acta 1402 (2026) 345395



Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca



Systematic optimization of gel-free capillary zone electrophoresis for phosphodiester oligonucleotide separations at acidic pH

Jakob Haglöf^{a,*}, Julia Regnell^a, Cari E. Säger-van de Griend^{a,b}

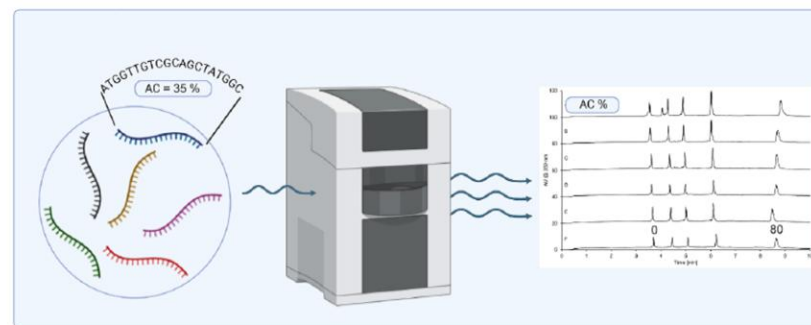
^a Department of Medicinal Chemistry, Uppsala University, Box 574, Uppsala, SE-751 23, Sweden

^b Kantisto BV, Haaksbergen, the Netherlands

HIGHLIGHTS

- Oligonucleotides are analyzed using acidic pH gel-free capillary zone electrophoresis.
- High resolution separations of same length oligonucleotides based on nucleobase pKa.
- Systematic optimization of the separation system for reproducible analyses.

GRAPHICAL ABSTRACT



<https://doi.org/10.1016/j.aca.2026.345395>

Latest publications by Kantisto

Electrophoresis

WILEY-VCH

ELECTROPHORESIS

RESEARCH ARTICLE

A Separation Buffers Platform Set to Facilitate CZE Charge Heterogeneity Method Development for Monoclonal Antibodies

Joanne Baxter¹ | Kevin Waltham¹ | Lori Fitton¹ | Cari E. Sanger-van de Griend^{2,3}

¹HB Innovations, Gateshead, UK | ²Kantisto BV, Haaksbergen, the Netherlands | ³Department of Medicinal Chemistry, Uppsala University, Uppsala, Sweden

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Keywords: ϵ -aminocaproic acid (eACA)-capillary zone electrophoresis (CZE) | analytical procedure control strategy | capillary zone electrophoresis | charge heterogeneity | critical materials | ICH Q14 | monoclonal antibody (mAb)

<https://doi.org/10.1002/elps.70089>

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- A Practical Approach to Implementing ICH Q14: Tools for Analytical Quality by Design in Capillary Electrophoresis Method Development Electrophoresis, 2025; 46:933–953
- Implementation of at-line capillary zone electrophoresis for fast and reliable determination of adenovirus concentrations in vaccine manufacturing
Electrophoresis, 2019; 40, 2277–2284
- One single, fast and robust capillary electrophoresis method for the direct quantification of intact adenovirus particles in upstream and downstream processing
Talanta 166 (2017): 8-14

