QbD for Upstream processing

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Imagination at work

Quality by Design

A framework for efficient process development

A systematic approach

Implementation of quality.
ICH guidelines

Quality cannot be tested into products
Quality has to be built in by design

ICH Q8
Pharmaceutical development

ICH Q9
Quality Risk Management

ICH Q10
Pharmaceutical Quality Systems

Why use Quality by design?

- Better understanding of product and process, increase robustness
- Reduce batch failures and reworks
- Drives quality systems

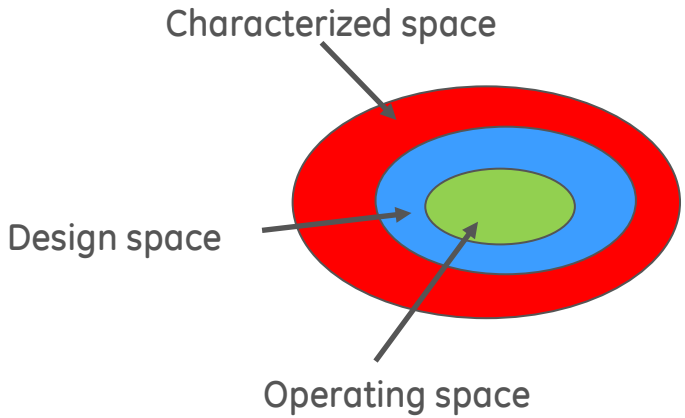
- Make use of historical knowledge
- Encourages innovation

- Simpler change management, understanding impact of future process changes

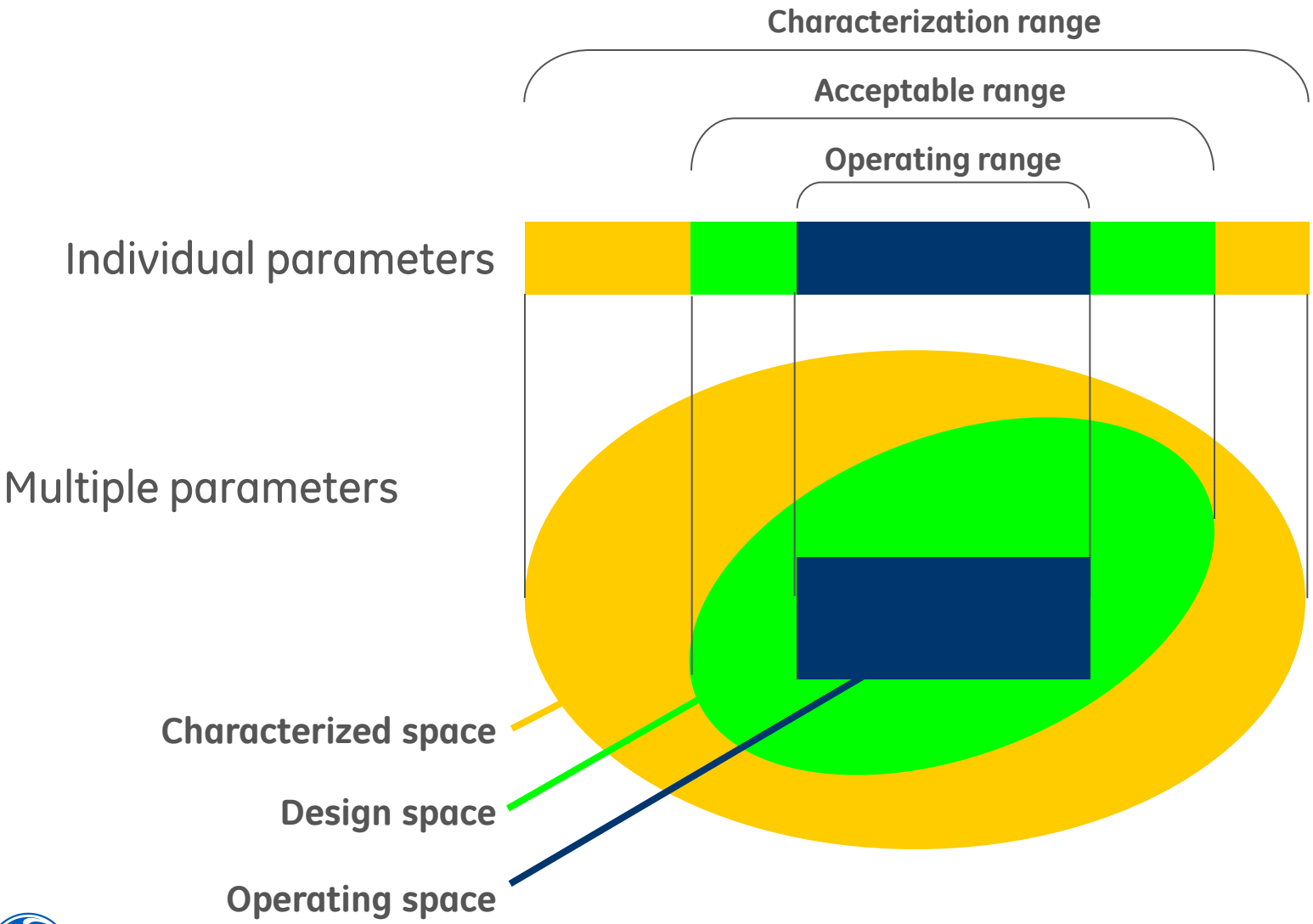
Process changes within the design space are not regarded as changes by the regulatory authorities

QbD workflow

- Identify potential sources of process variability
- Identify parameters likely to have greatest impact on product quality
- Design and conduct studies to identify relationships of material quality and process parameters to Critical Quality Attributes (CQA)
- Analyse and assess data to establish appropriate ranges including design space



Design Space is multi-dimensional



Process design space

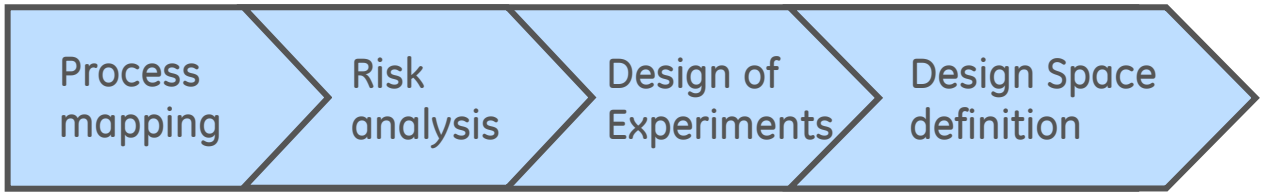
ICH Q8(R2), Pharmaceutical Development

- ✓ The multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality
- ✓ Working within the design space is not considered as a change of the approved ranges for process parameters
- ✓ Movement out of the design space is considered to be a change and would normally initiate a regulatory post-approval change process

QbD workflow:
Defining the process design space

Four key steps

1. Process mapping
2. Risk analysis
3. Design of experiments (DoE)
4. Execution and analysis, definition of design space

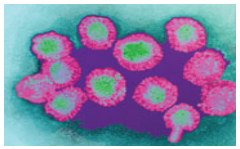


Vaccines and QbD

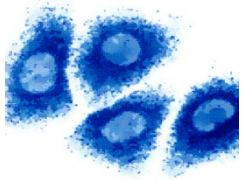
Can QbD be used for Vaccines?

The Vaccines

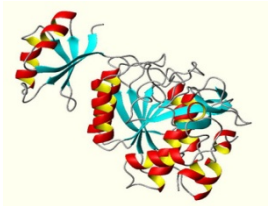
Bacteria based



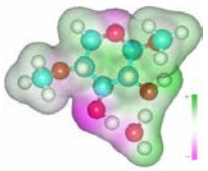
Virus based



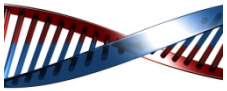
Protein based



Polysaccharide based



DNA based



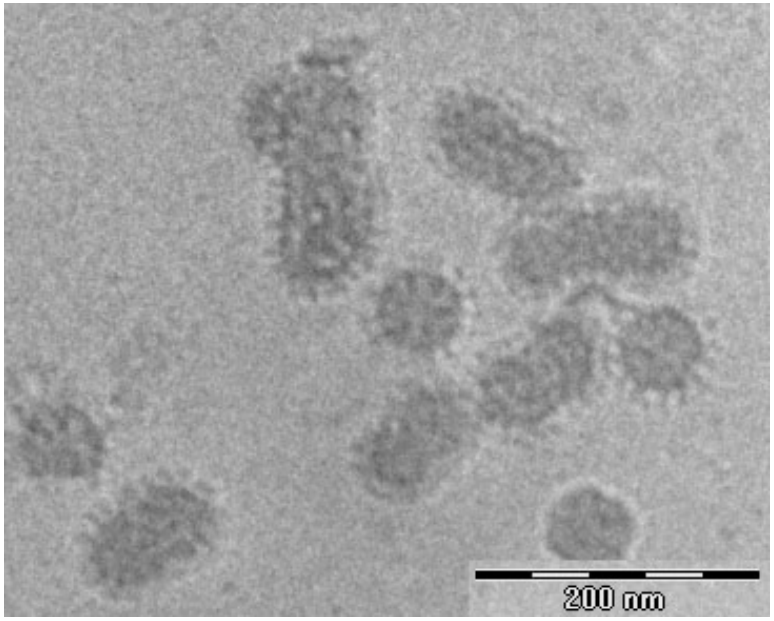
Challenges

- Not regarded as well characterized
- Processes often not so well defined, no platform processes
- Analytical assays with low precision

Influenza vaccines

Influenza virus

- Viral infection that causes respiratory disease
- 3-5 million cases of severe illness and >300 000 deaths annually
- Severe economical consequences
- 3 pandemics in the 20th century



Cryogenic Transmission Electron Microscopy of influenza A/Puerto Rico/8/1934 (H1N1)

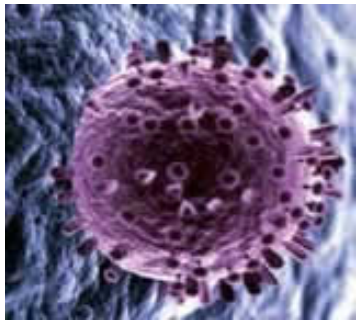
- 3 types: A, B and C
- Lipid envelope, two major membrane-bound glycoproteins:
  - Haemagglutinin (HA)
  - Neuraminidase (NA)

Live attenuated Influenza vaccines

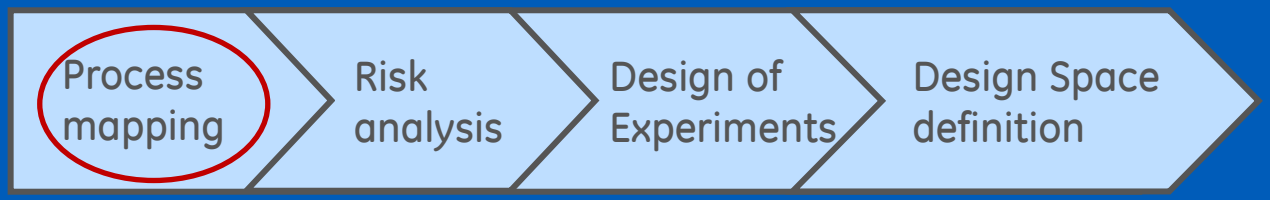
- Cold adated influenza virus strains
- Approved in certain markets
- Produced in eggs, cell based variants in development
- Low dose size
- Low production volumes/dose

QbD considerations for live attenuated influenza virus preparation:

Dose	$10^{6.5}$ -$10^{7.5}$ infectious particles/0.2 ml
Residual DNA	< 10 ng/dose
Total protein	< 300 µg/dose
Intact cells	absence of
Hemagglutinin, HA	to be correlated with TCID (Tissue culture infective dose)



Process mapping

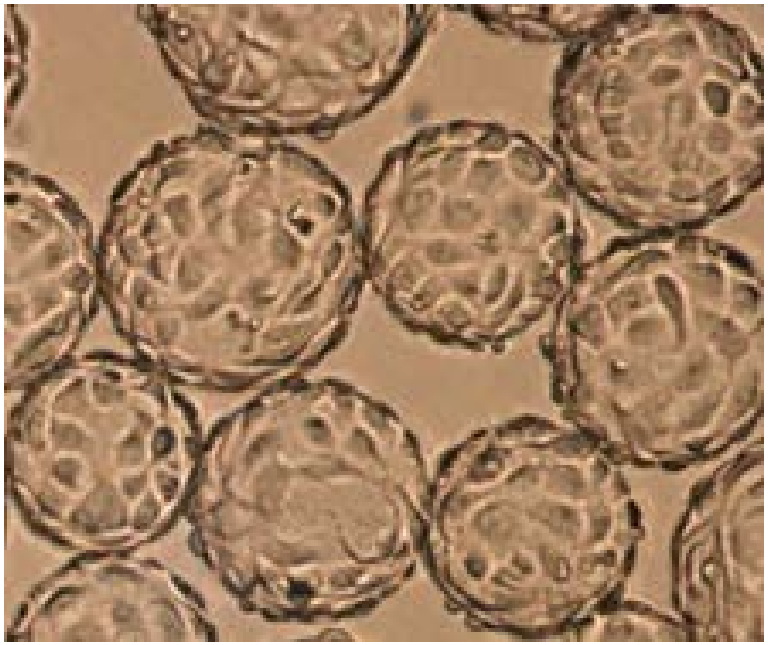


QbD workflow:
Defining the process design space

1. Process mapping
   - Which factors could potentially affect our process?
   - Tools: High level process maps
     Fishbone diagrams

Cell substrate for Influenza virus production

- Modern options: **Vero or MDCK**
- Anchorage dependent, can be expanded on Cytodex™ microcarries
- Animal origin free cell culture medium
- Cell line requirements
  - Suitable for GMP production
  - Good safety track record
  - Good virus propagation
  - Broadly and highly permissive
  - Scalable to high volume production



Influenza process overview –

High level process map

Upstream

Cell inoculum
From static cell factories

Seed culture (10 L)
WAVE Bioreactor™ 20/50 system

Cell transfer
Bead to bead transfer

Production culture (50 L)
WAVE Bioreactor 200 system

Downstream

Clarification
ULTA™ Prime GF

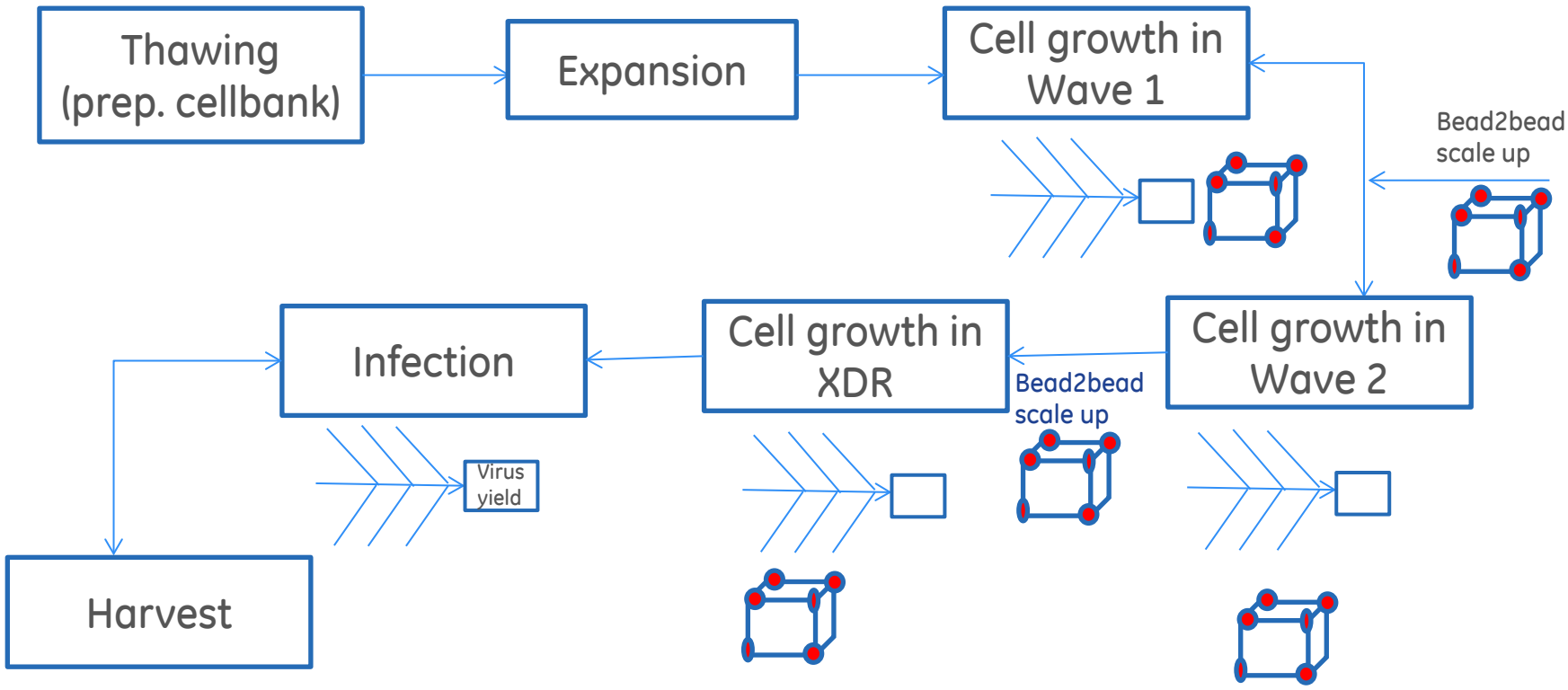
Chromatography
ÄKTA™ ready system

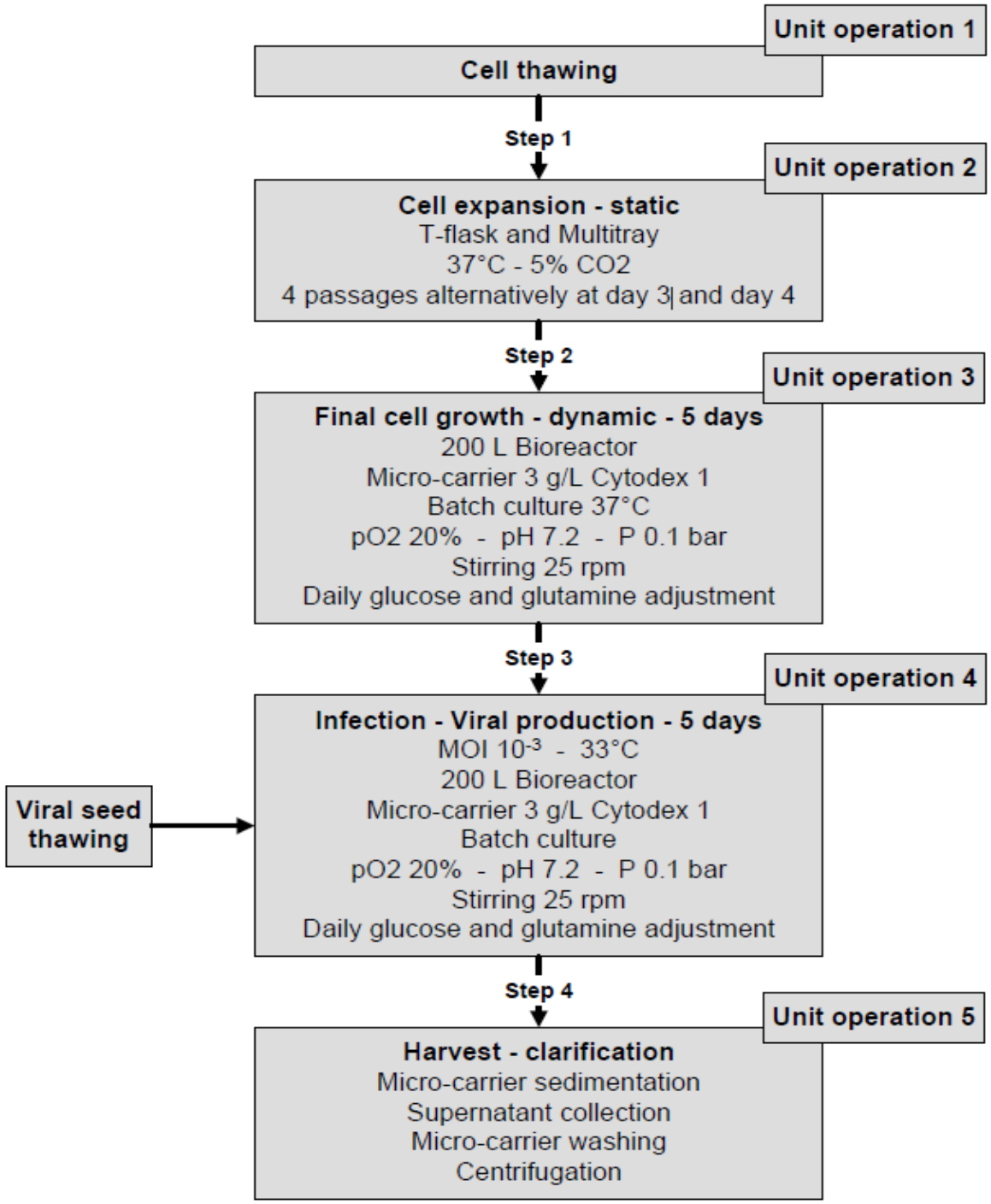
Concentration and buffer exchange
ReadyCircuit™ assemblies

Sterile filtration
ULTA Pure HC

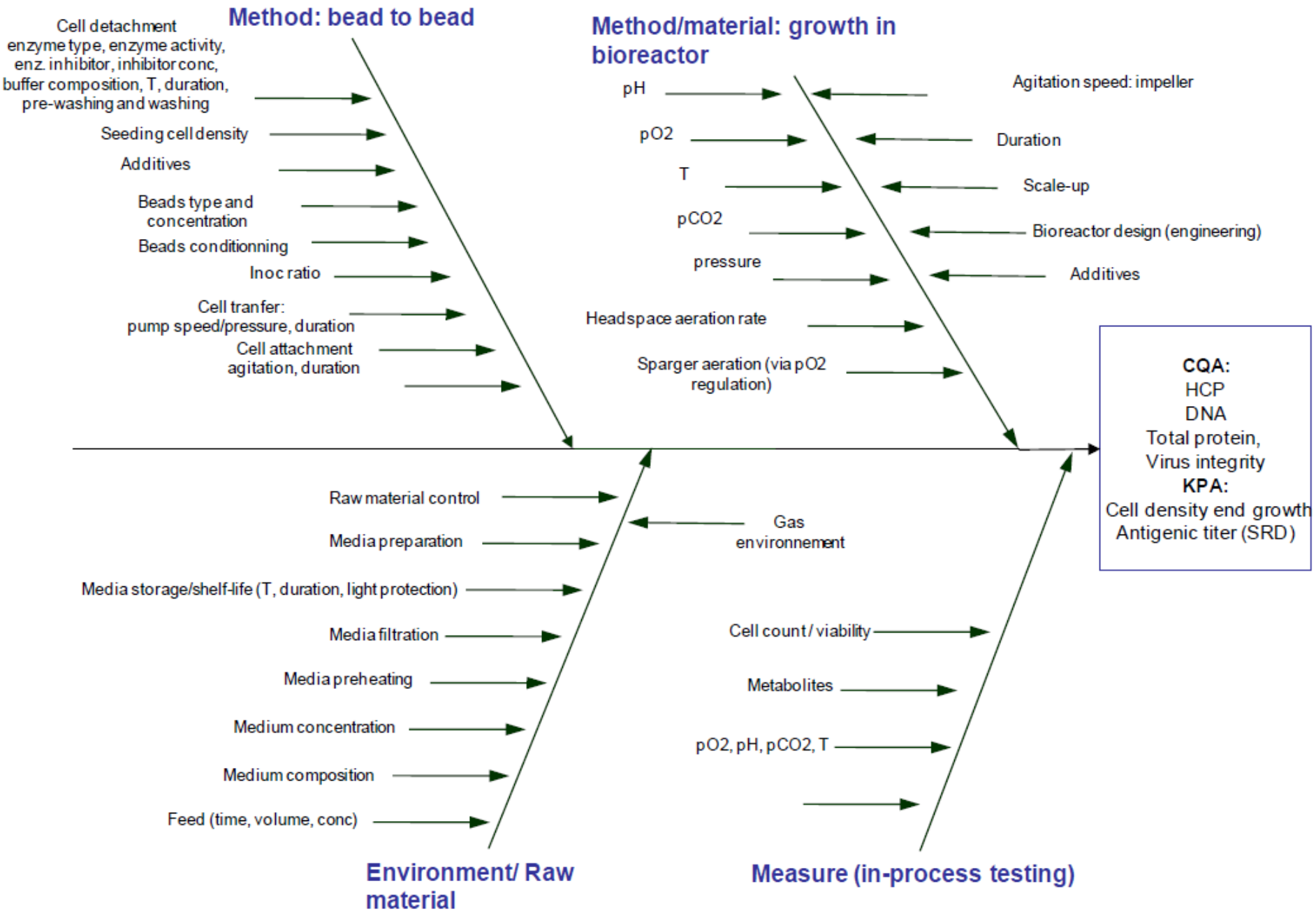
Virus analysis
Biacore™ T200 and other methods

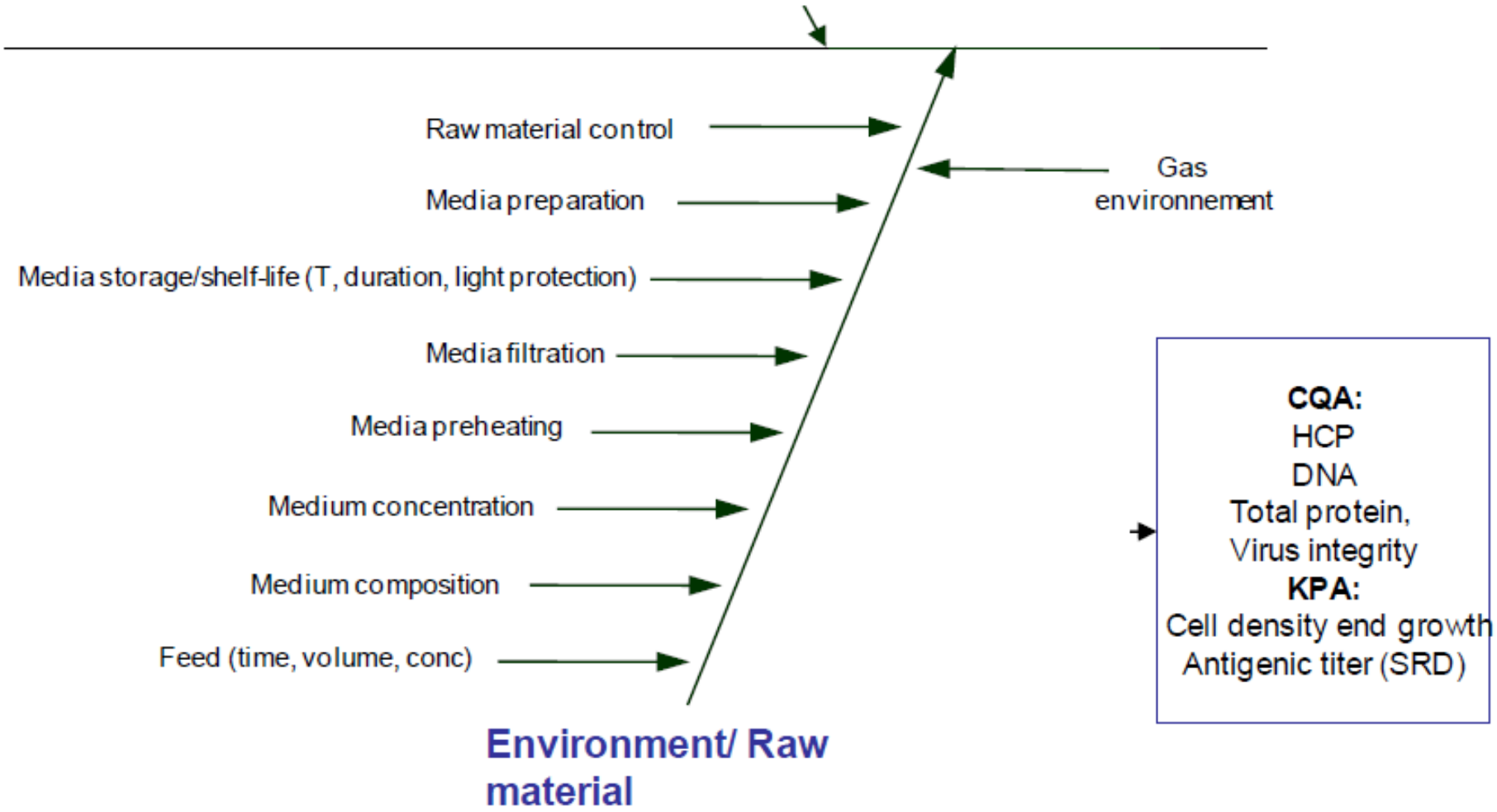
QbD in Influenza vaccine upstream



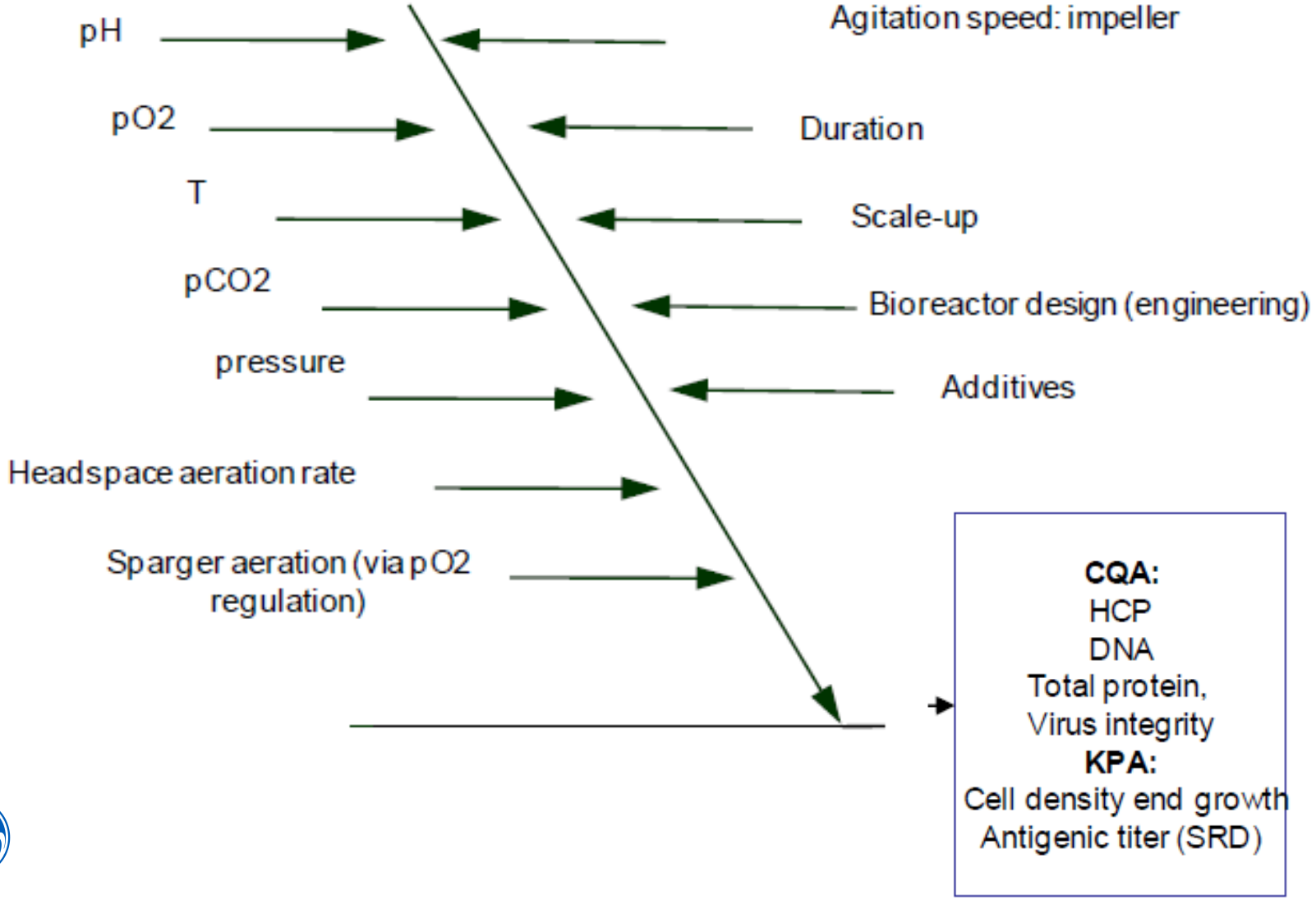

Unit operation 1
Cell thawing
Step 1
Unit operation 2
Cell expansion - static
T-flask and Multitray
37°C - 5% CO2
4 passages alternatively at day 3 and day 4
Step 2
Unit operation 3
Final cell growth - dynamic - 5 days
200 L Bioreactor
Micro-carrier 3 g/L Cytodex 1
Batch culture 37°C
pO2 20% - pH 7.2 - P 0.1 bar
Stirring 25 rpm
Daily glucose and glutamine adjustment
Step 3
Unit operation 4
Infection - Viral production - 5 days
MOI 10-3 - 33°C
200 L Bioreactor
Micro-carrier 3 g/L Cytodex 1
Batch culture
pO2 20% - pH 7.2 - P 0.1 bar
Stirring 25 rpm
Daily glucose and glutamine adjustment
Viral seed thawing
Step 4
Unit operation 5
Harvest - clarification
Micro-carrier sedimentation
Supernatant collection
Micro-carrier washing
Centrifugation

Final growth on microcarriers

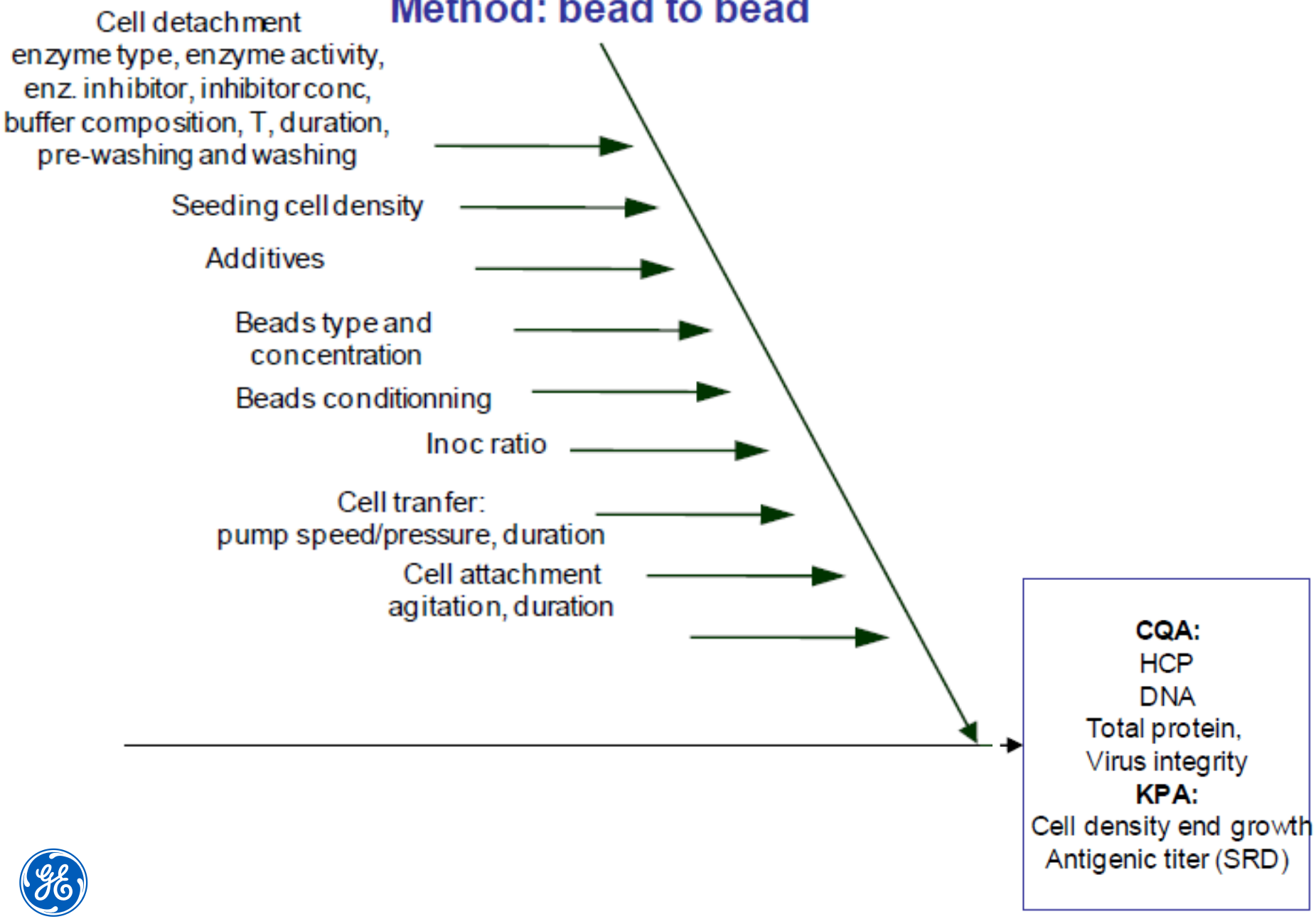


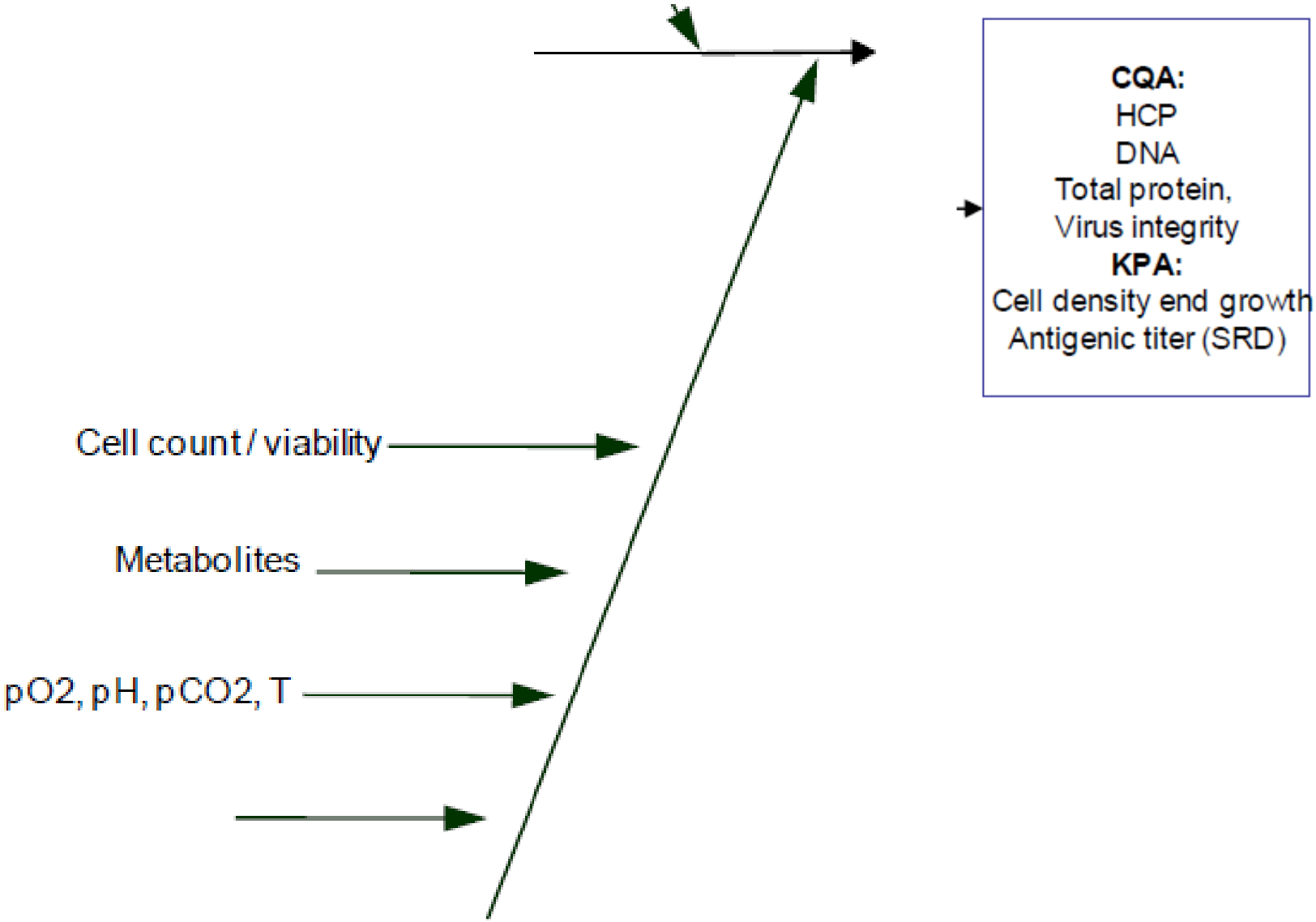

Raw material control
Media preparation
Media storage/shelf-life (T, duration, light protection)
Media filtration
Media preheating
Medium concentration
Medium composition
Feed (time, volume, conc)
Gas environnement
CQA:
HCP
DNA
Total protein,
Virus integrity
KPA:
Cell density end growth
Antigenic titer (SRD)
Environment/ Raw material

Method/material: growth in bioreactor



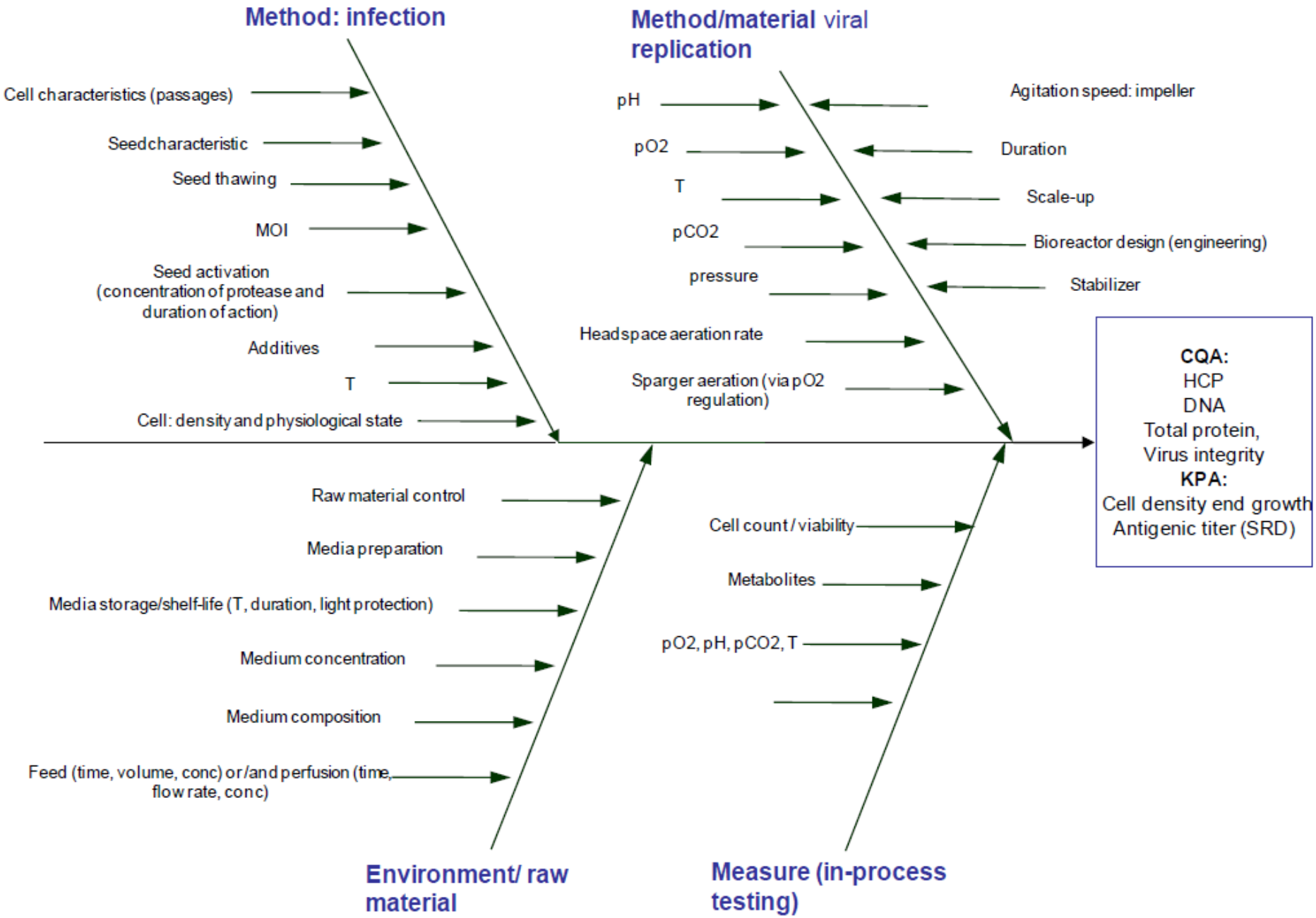
Method: bead to bead



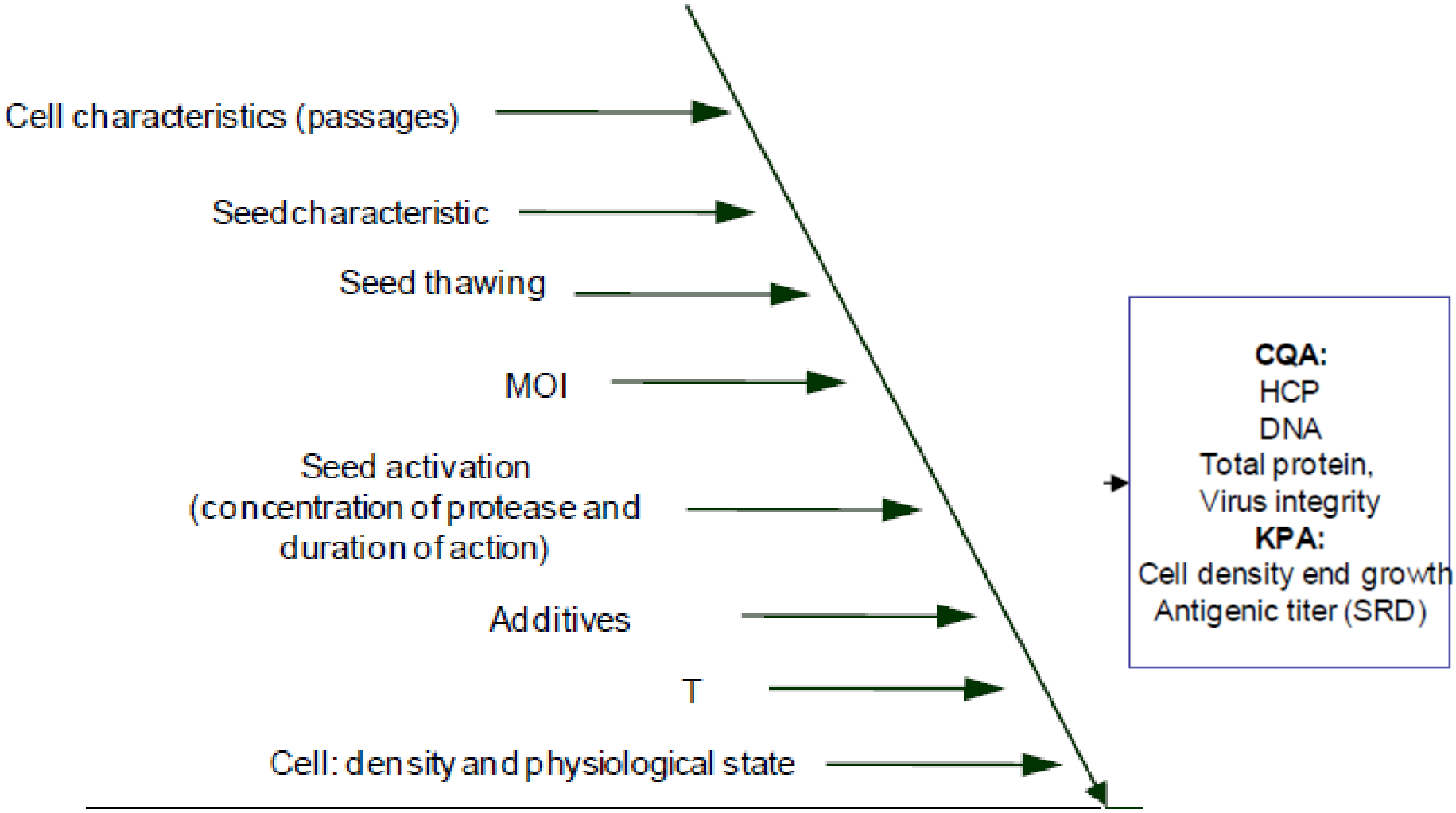


Measure (in-process testing)

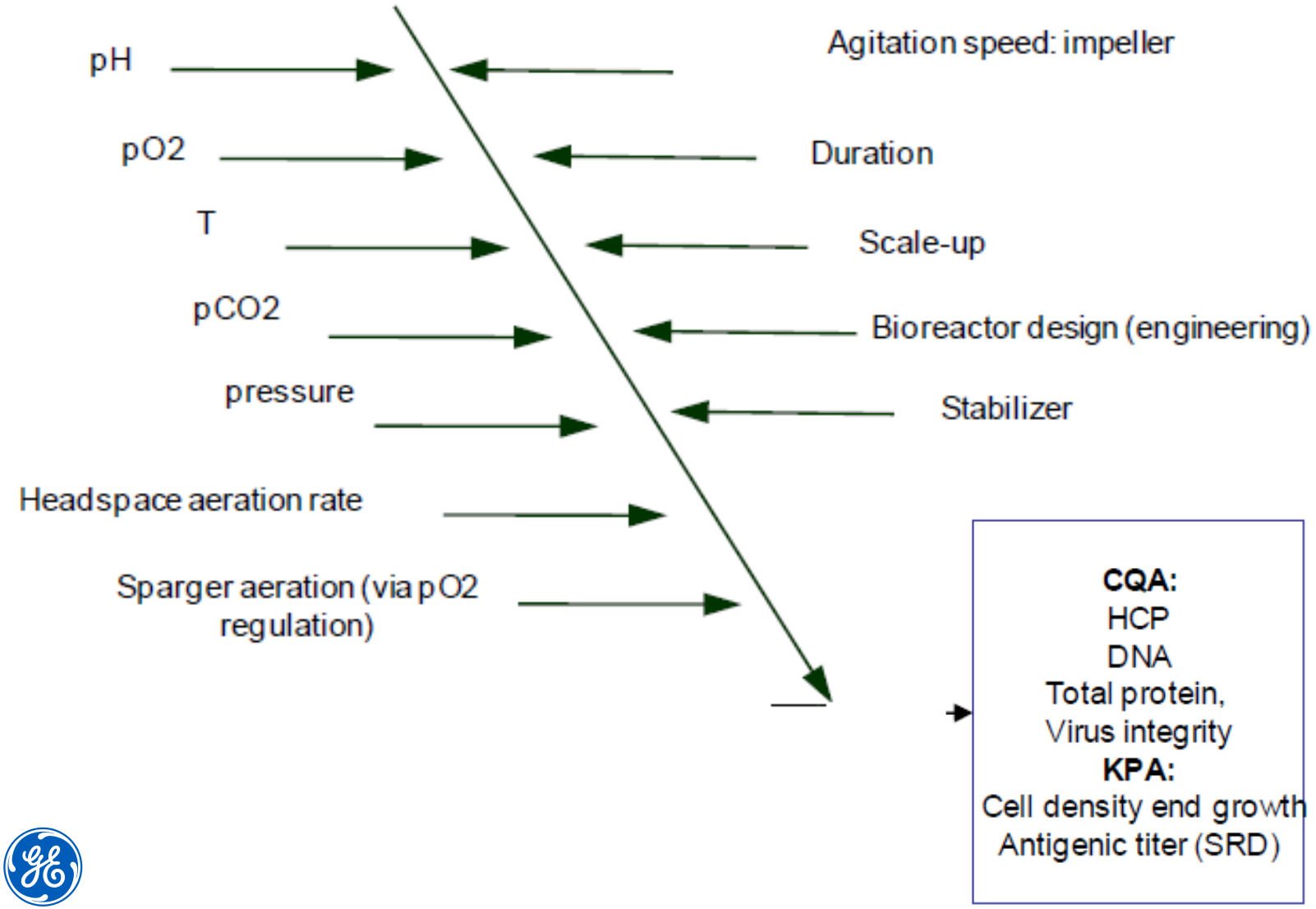
Infection – viral production



Method: infection



Method/material viral replication



Risk analysis and actions

QbD workflow:
Risk analysis, FMEA

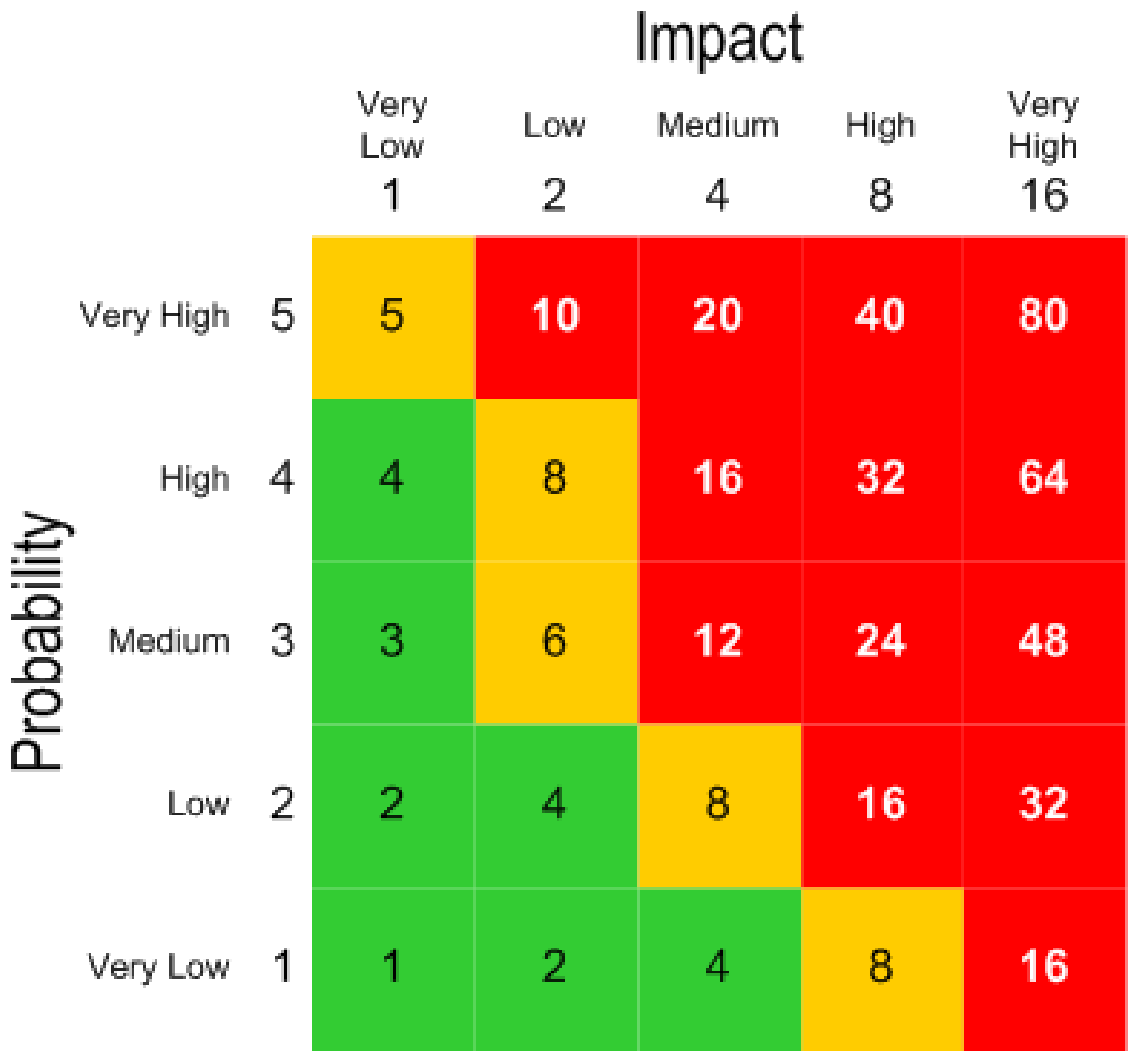
2. Risk analysis and actions
   - Which parameters should be investigated in detail?
   - Tool: Failure Mode and Effects Analysis (FMEA)

<u>Ranking of effects</u>
What might go wrong?
How severe are the consequences?
What is the likelihood the failure occurs?
Can we detect the failure?

Risk score

Cross functional involvement is important
R&D, Process dev., Manufacturing, Sourcing, QA, QC etc

QbD workflow:
Risk analysis, FMEA



QbD workflow: Risk analysis, FMEA

S - Severity of each failure (1-10)
O - Likelihood of occurrence (1-10)
D - Likelihood of detection (1-10)
Risk Score = S x O x D

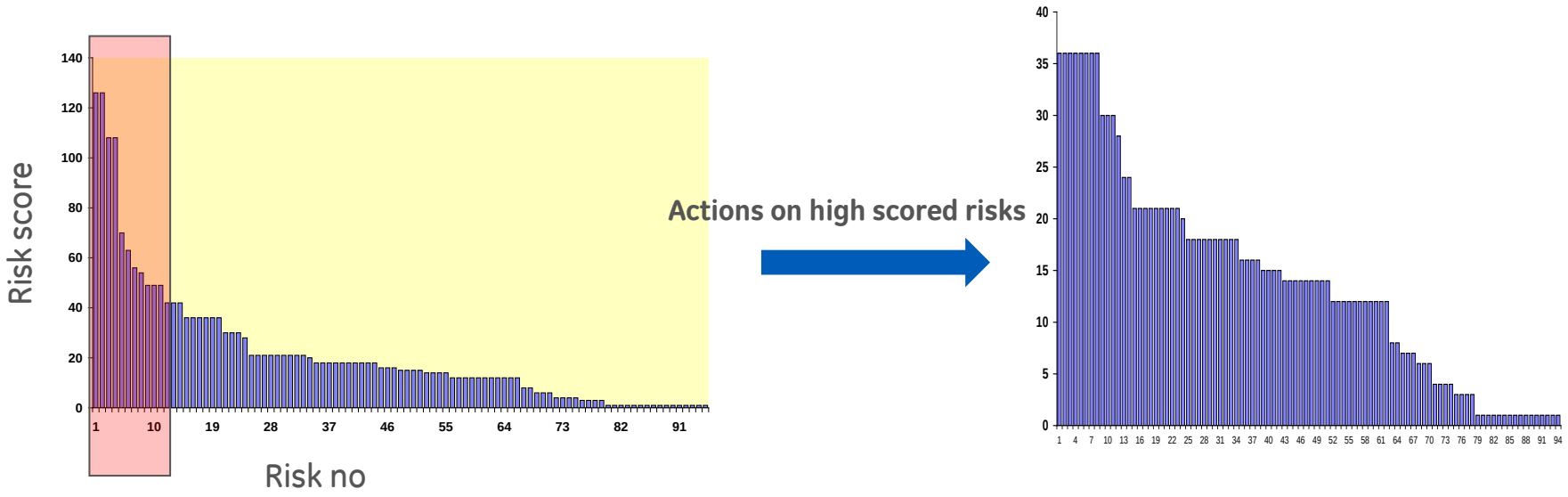
Define what each level represent

Level	Severity (S)	Occurrence (O)	Detection (D)
9-10	Possible harm/injury to patient or operator	Every batch/run	Will probably not be detected by existing systems
7-8	Loss of several batches, damage to equipment	Once in 2-10 batches	Detection at batch release or at periodical control after batch completion
5-6	Batch involved probably lost/needs to be discarded	Twice yearly, ~once every 10 batches	Detection at batch release/equivalent
3-4	Small consequences: Additional batch testing, re-work...	Once yearly/ once in every 50 batches	Will be detected at-line before next unit operation by existing systems
1-2	No or negligible consequences	Very low/Practically no occurrence	Immediate, obvious detection

QbD workflow:
Risk analysis, FMEA score card

Item / Process Step	Potential Failure Mode(s)	Potential Effect(s) of Failure	S	Potential Mechanism(s) of Failure	O	Potential Cause(s) of Failure	Current Process Controls	D	RPN	Recommended Action(s)	Action Results: Actions Taken	S	O	D	RPN
Sample conc.	Above limit	Low purity	6	Analysis error	3	Operator, dilution	No controll	7	126	Orthogonal analysis & 2 analytical personel	Yes	6	2	2	24
Sample amount	Below limit	Low yield	6	Analysis error or operator error	3	Operator, dilution	No controll	7	126	Orthogonal analysis & 2 analytical personel, analysis after dilution, double check calculations & Double check methods or test run	Yes	6	2	2	24
System assembly	Wrong flow path	Multiple	9	Multiple	3	Operator	Visual inspection	4	108	Double ckeck set up and test run	Yes	9	2	2	36
System failure	Multiple possibilities	Multiple	9	Multiple	3	System failure	None	4	108	Service routine/contrat and test runs	Yes	9	2	2	36
CIP/Strip volume	Below limit	Risk of carry over/Build up	7	Insufficent cleaning	1	Operator/instrument	No controll	10	70	Check pH and volumes, in/on-line TOC analysis	Yes	7	1	1	7
Sample load	Above limit	Low purity	7	Wrong sample amount applied	3	Operator	at line	3	63	Verification by second operator or fixed volume of sample	Yes	7	1	2	14
CIP cond	Above limit	Loss of media	8	Buffer preparation	1	Operator/instrument	In line	7	56	Doble check conductivity of CIP solution	Yes	8	1	2	16
CIP cond	Above limit	Low purity	7	Loss of capacity/Ligand	1	Operator	In line	7	49	Doble check conductivity of CIP solution	Yes	7	1	2	14
CIP cond	Above limit	Low yield	7	Loss of capacity/Ligand	1	Operator	In line	7	49	Doble check conductivity of CIP solution	Yes	7	1	2	14

QbD workflow:
Risk analysis plot

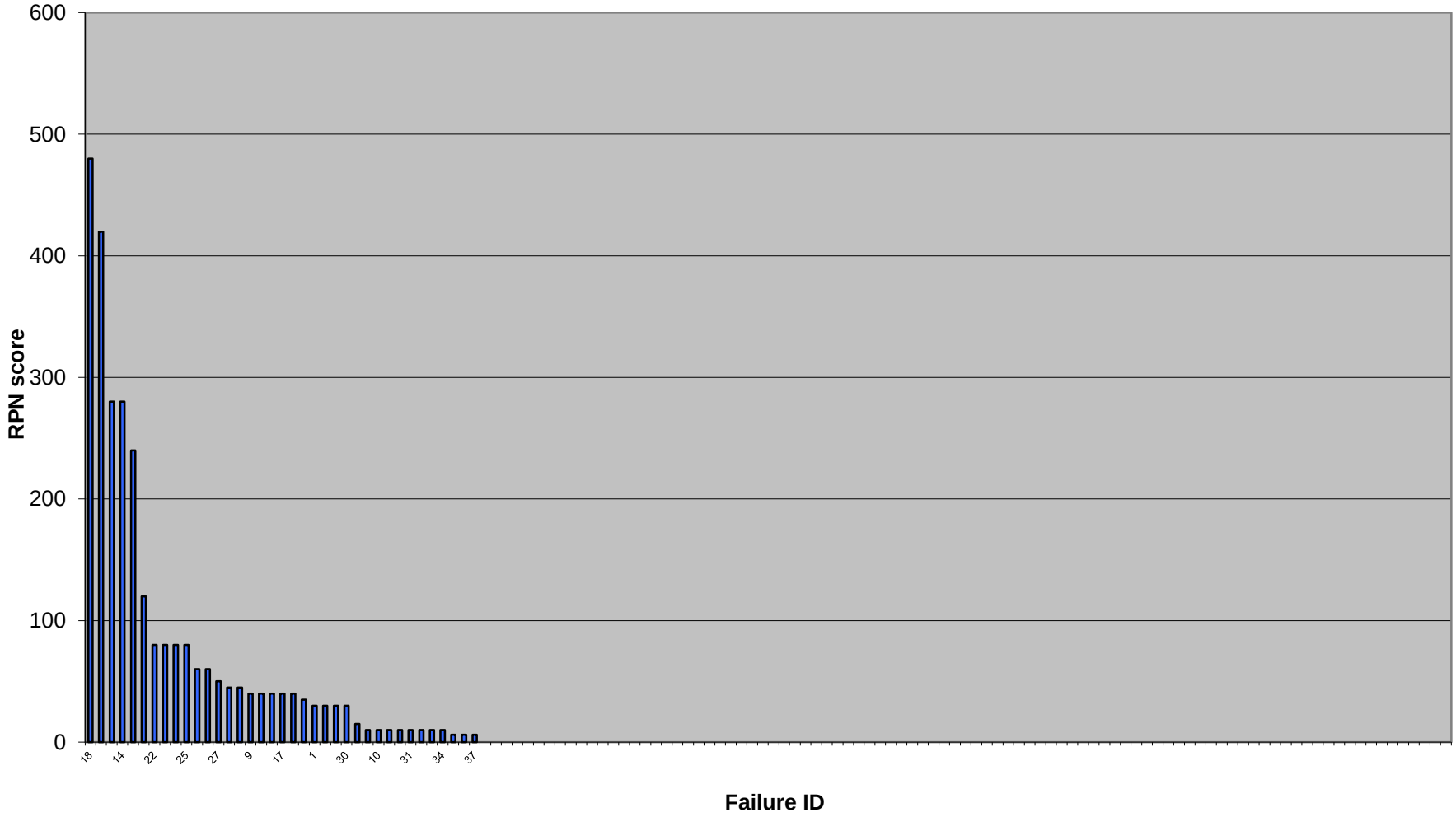


Identification of critical unit steps/critical unit step parameters for further examination, i.e. DoE

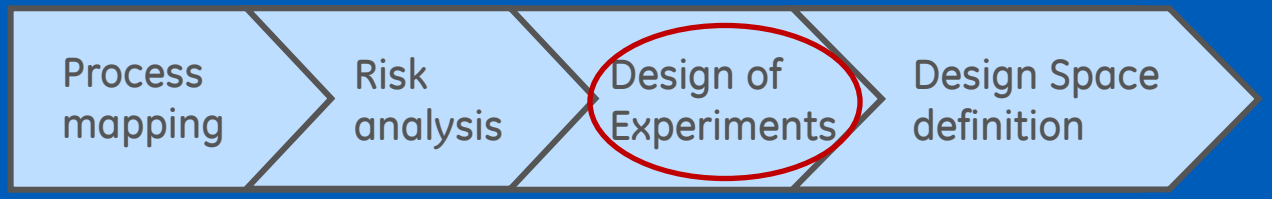
Failure Mode and Effects Analysis (Process FMEA) Cell Bank

		What is the defect?	What is the impact on the process customer?		What causes the defect?		Sources of Variability - (think Fishbone)			
Item / Process Step	Potential Failure Mode(s)	Potential Effect(s) of Failure	Sev	Potential Mechanism(s) of Failure	Occur	Potential Cause(s) of Failure Mechanism	Current Process Controls	Det	RPN
Cell Bank									
Freezing medium	DMSO low	low Viability	6	operator failure	1	stress, mis calculation	viability check	1	6
	DMSO high	low Viability	6	operator failure	1	stress, mis calculation	viability check	1	6
	serum not included	low Viability	6	operator failure	1	stress, mis calculation	viability check	1	6
	wrong medium	low Viability	6	operator failure	1	stress, mis calculation	viability check	1	6
	contamination	bank unusable	8	raw material contaminated	1	manufacturer	no	10	80
	contamination	bank unusable	8	laf bench failure filter	1	service	no	10	80
	contamination unknown	bank unusable	8	operator failure	1	stress	no	10	80
Storage	N2 min limit reached	bank unusable	8	operator failure	1	routines, communication	no	10	80
	electrical failure (freezer)	bank unusable	8	equipment malfunction	4	power outage	alarm and service	1	32
Thawing protocol	too fast	low Viability	4	operator failure	2	water temperature high	viability check	1	8
	too slow	low Viability	4	operator failure	2	water temperature low	viability check	1	8
	wrong size of T-flask	low Viability	4	operator failure	1	wrong cell concentration	SOP	1	4
	no DMSO removal	low Viability	4	operator failure	1	stress	viability check	1	4
	contaminated cell bank	bank unusable	8	operator failure	3	insufficient aseptic handelling	sterility test	1	24
	contaminated cell bank mycoplasma	bank unusable	8	operator failure	2	stress	mycoplasma test	1	16
Freezing	centrifugation speed high	low cell number due to cell death	6	operator failure	1	xg/rpm	no	5	30
	centrifugation speed low	low centrifugation recovery	2	operator failure	1	xg/rpm	pellet size / opaque supernatant	1	2
	cell density low	low Viability	5	cell counter malfunction	2	no service, insufficient maintenance	no	5	50
	cell density low	low Viability	5	operator failure	2	calculation error	no	5	50
	inhomogeneous cell suspension	variable quality	7	operator failure	3	insufficient mixing	no	8	168
	uncontrolled freezing conditions	low Viability/cell death	6	operator failure	1	forget to add iso-propanol	min limit mark	1	6
	contamination	bank unusable	8	operator failure	3	insufficient aseptic handling	no	5	120
	cells in plateau phase	low viability	5	operator failure	3	cell density too high	cell counting	1	15
	passage number too high	thawing of new vial more often	3	operator failure	2	lack of experience	passage number check	1	6
Purchase of new cell line	contaminated ampoule	bank unusable	8	manufacturer failure	3	manufacturer routines	sterility test	2	48
Size	low number of vials	variation in quality / increased work load	3	operator failureQbD Vaccines 2013-03-18_TinaG	1	lack of experience	no	8	24

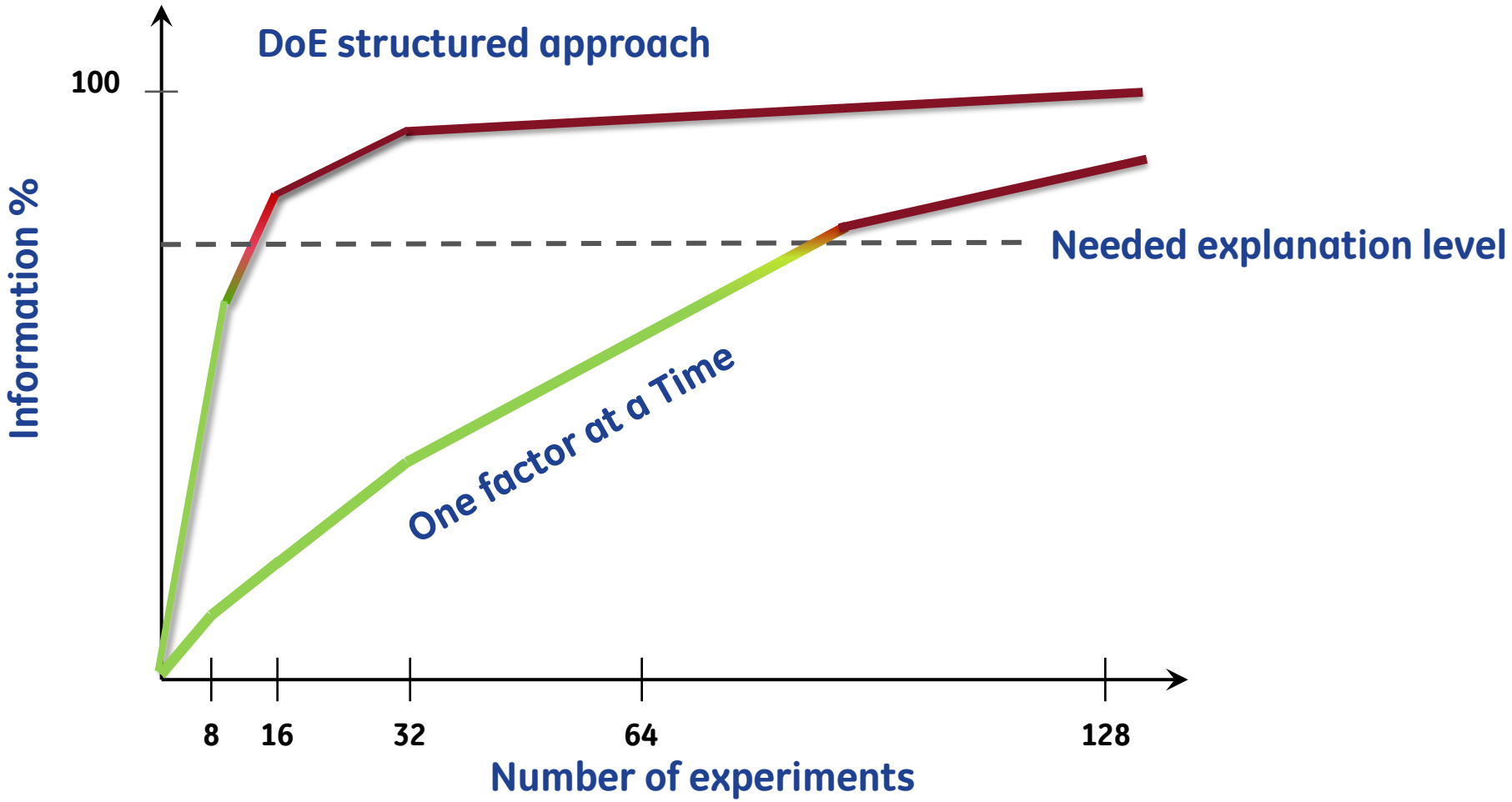
Pareto plot Bioreactor conditions



Design of Experiments



QbD workflow:
Design of Experiments (DoE)



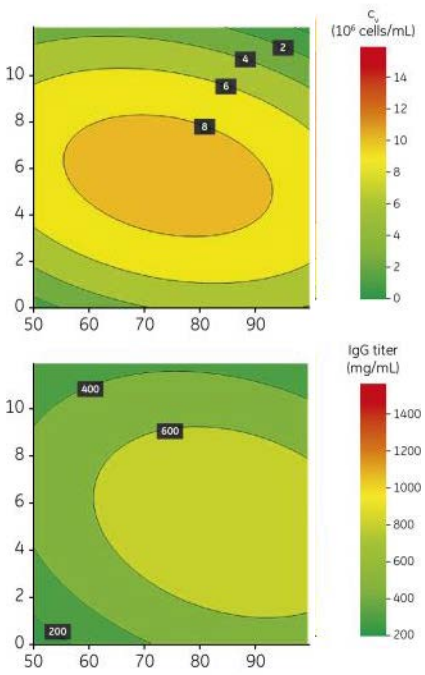
DoE can be helpful to get maximum information from a minimal number of experiments

DoE summary

- Powerful tool when examining moderately high number of points and parameters
- Now contained in better commercial software packages
- Has limitations with very small or very large or very complex data sets
- Will not address assay imprecision / inaccuracy
- Relies upon operator judgment on input and iterative parameter set selection

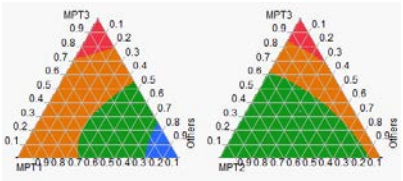
DoE in media and process design

- Not magic
- Input data limits output
- Experimental scale / control factors in experiments
- Examine contour plot below
  - Could you find a solution from raw data?

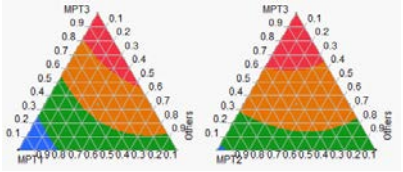


Mixture design points to optimal formulation

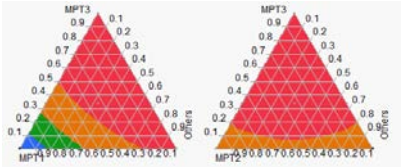
- DoE mixture design greatly improves growth characteristics
- Selection of basal media through screening of reference formulations
- DoE simplex lattice mixture design study using top four media (total 28 conditions)
- Growth curve and viability profiles evaluated for optimal formulation
- PVCD and IVCA generated for each condition and compared to product quantity
- Ternary plots of DoE mixture design prototype conditions. "Hot Spots" (red) on these plots show that mixtures higher in media prototype 3 (MPT3) from the initial screening yielded higher product levels



Product levels



IVCA



PCD

Ternary plots resulting from simplex lattice mixture design

Examples of DoE studies in cell culture PD

Study	Factors	Comments
Cell substrate selection	Screen of virus productivity	Select the optimal substrate
Cell culture media	Different media Supplements and additives	Determination of optimal concentrations
Physical conditions	Agitation, pH, O2/CO2, temperature	Operating conditions
Microcarrier culture	Cell densities Attachment conditions Bead to bead scale-up	Applicable for anchorage dependent cells
Virus propagation	Virus activation, Multiplicity of infection (MOI), Time of infection (TOI),	Virus activation applicable for certain viruses that need activation by enzymes such as trypsin
Harvest	Time of harvest (TOH)	

Culture formats for DOE:

	Plates	Mini bioreactors	Standard bioreactors
Number of cultures	Very high	Medium	Low
Culture volume	Low	Low	Larger
Factor screening	Broad	Broad	OFAT[1]
Sensor options	No	Limited	Yes
Automation	Manual Robot	Manual Robot	Bioreactor system

1) OFAT = one factor at a time

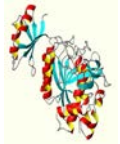
Analytics for Influenza Virus Vaccines

Analytics in DoE

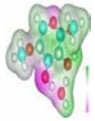
Bacteria based

Virus based

Protein based



Polysaccharide based



DNA based

Challenges in general

- Large number of tests during development
- Sensitivity and precision is critical
- HTPD* methods ”creates” variable sample matrixes -> effects on analytical methods
- Miniaturization and parallelization puts higher demands on analytical method sensitivity and capacity

Challenges for vaccines

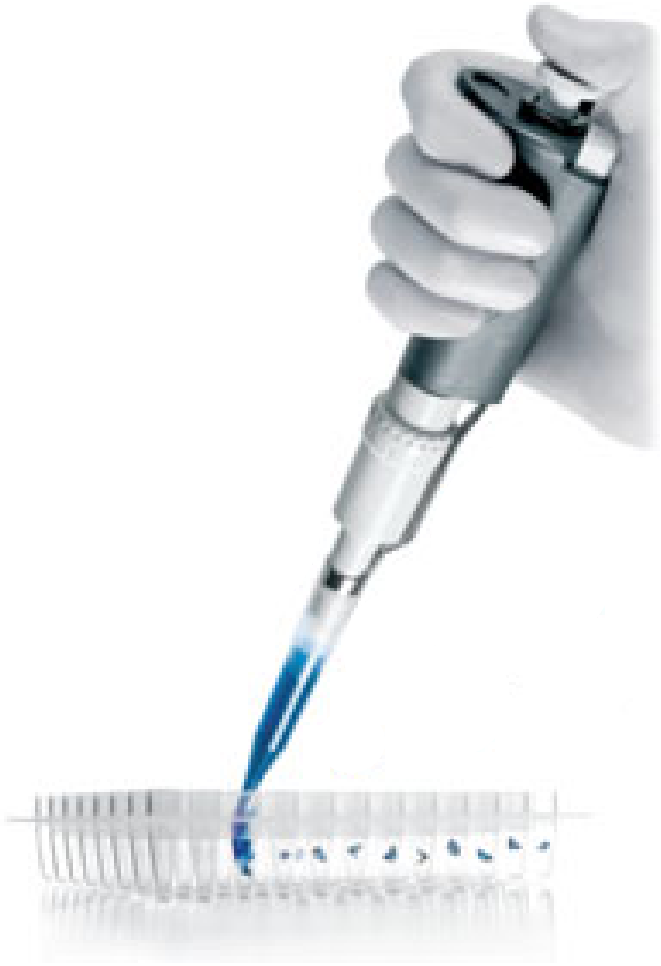
- Not regarded as well characterized
- Processes often not so well defined
- Analytical assays with low precision
- Time consuming, SRID, $TCID_{50}$

*High throughput process development

Analytical tools - a bottleneck

Typical analytical challenges during vaccine development and production:

- **Hundreds of tests** per run in both development (DoE) and production
- **Time consuming** to complete analysis, particularly in-vivo testing
- Varying **uncertainty** in test accuracy



Analytical tools in influenza vaccine manufacturing

High quality analytical tools are required to qualitatively and quantitatively measure the recovery, yield and purity of the virus

Presently used methods

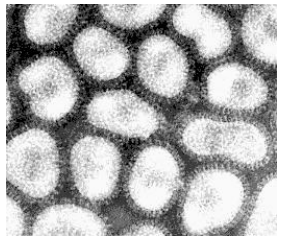
- Single radial immunodiffusion (SRID)
- Haemagglutinin (HA) agglutination assay
- HA enzyme-linked immunosorbent assay (HA-ELISA)
- Western Blot/Dotblot
- TCID50

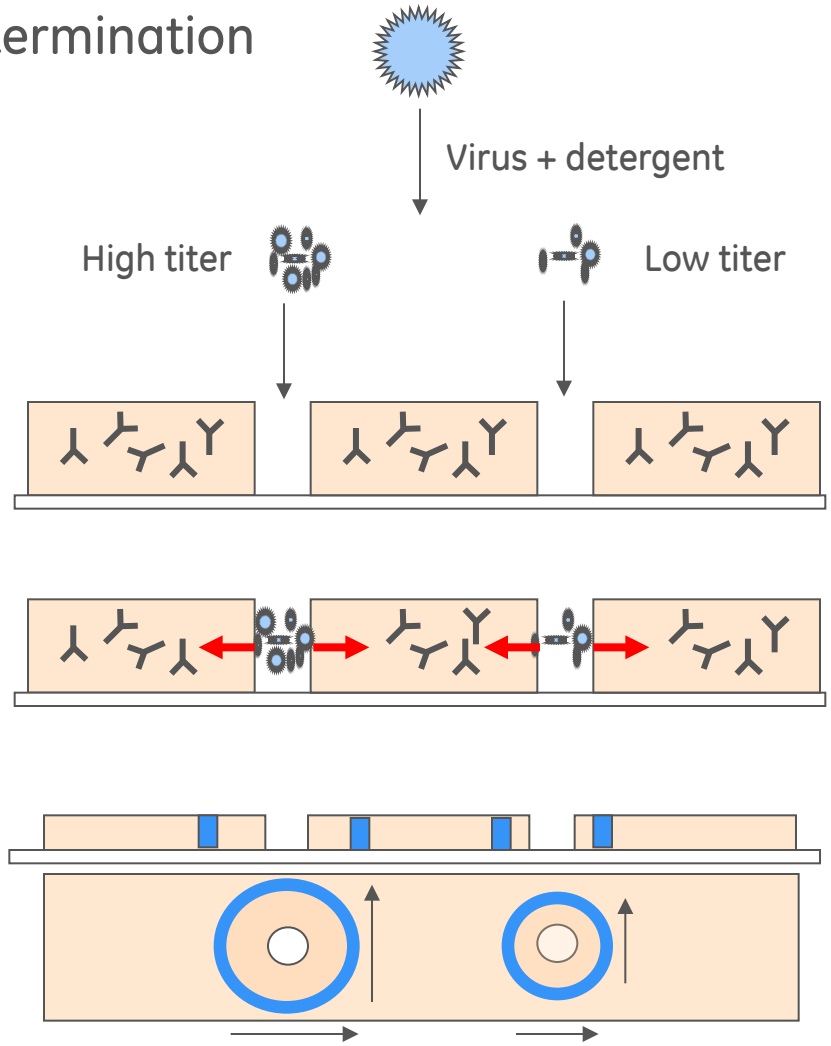
Challenges

– Sensitivity, detection range
– Method variation (Precision, Accuracy)
– Hands on time
– Cost
– Robustness
– 3 influenza strains;
A/H1N1, A/H3N2 and B

Single radial immuno diffusion (SRID)

Virus titer determination





Agarose gel with antibodies (Ab)
Holes punched in gel

Sample with virus antigen (Ag) added

Antigen diffuses in to the gel
Ab-Ag precipitation forms 18-24h

Gel washed, dried and stained (Coomassie)

Ring area measured
Compared to known reference

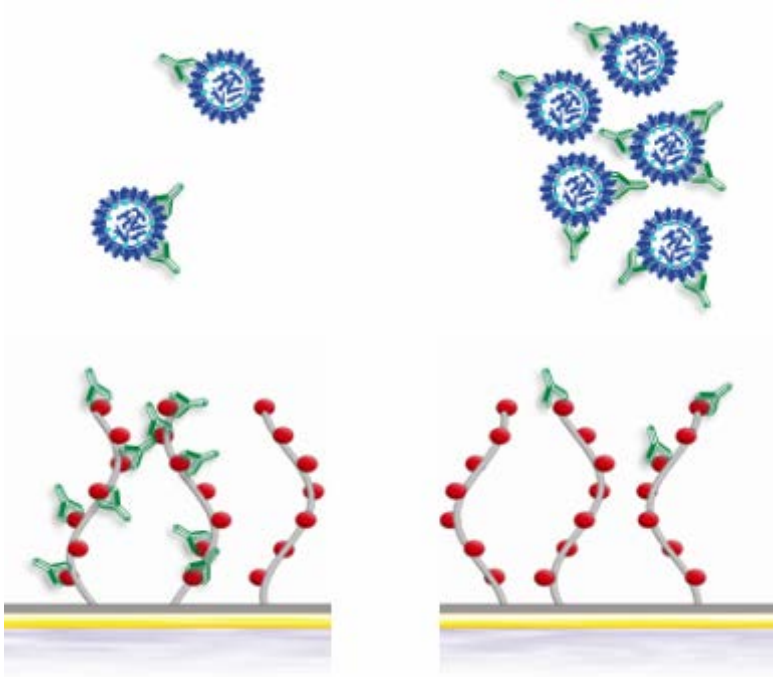
Biacore™ assay setup

Inhibition assay

Anti-HA-Ab binding to:

The virus in the sample

Influenza HA proteins immobilized on the surface

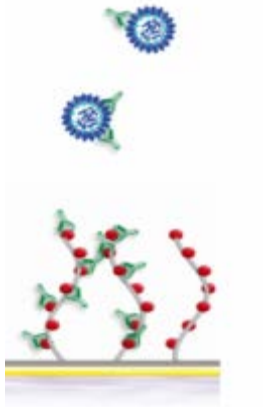


Concentration of virus	LOW	HIGH
Response from surface	HIGH	LOW

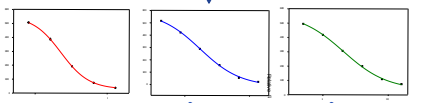
Ref: Estmer Nilsson *et al*. 2009. A novel assay for influenza virus quantification using surface plasmon resonance. Vaccine 28, p.759-766.

Quantification of a 3 strain seasonal influenca vaccine

Biacore™assay



Final TBV

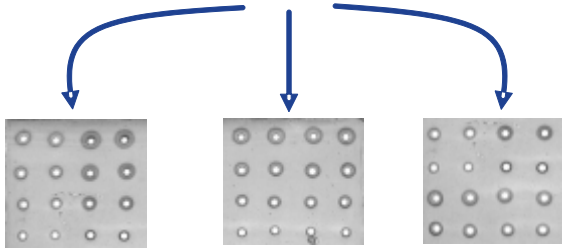


B A/H1N1 A/H3N2

Hemagglutinin

Single radial immunodiffusion (SRID)

WHO Pharmacopeia



B A/H1N1 A/H3N2

Assay time: 100 samples, incl. controls & standards

	1 multiplexed assay	3 separate assays
Hands on time	**1-2h**	**6-8 h**
Total analysis time	**15-16h**	**20-22 h total**
LOQ	**1 µg/ml**	**12 µg/ml**
Precision (# samples with CV<5%)	**95%**	**18%**

Summary Biacore vs SRID

	Biacore™	SRID
dynamic range		
(µg HA/mL)	0.5-10	5-20
sensitivity		
LOD (µg HA/mL)	0.5	5
LOQ (µg HA/mL)	1.0	10
precision		
(%) samples CV < 5%	97	18
recovery		
(%)	95-105	90-110
time for 100 samples		
hands-on (hours)	1 - 2	6 - 8
Total (hours)	15 - 16	20 - 22

Ishikawa diagram