QbD for Upstream processing

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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

Characterization range
Acceptable range
Operating range

Individual parameters
Multiple parameters

Characterized space
Design space
Operating space
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?

- 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

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

Cryogenic Transmission Electron Microscopy of influenza A/Puerto Rico/8/1934 (H1N1)
Live attenuated Influenza vaccines

- Cold adapted 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 \(\mu\)g/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
QbD in Influenza vaccine upstream

Thawing (prep. cellbank) → Expansion → Cell growth in Wave 1

Infection → Cell growth in XDR

Harvest → Virus yield → Cell growth in Wave 2

Bead2bead scale up
Final growth on microcarriers

Method: bead to bead
- Cell detachment
  - enzyme type, enzyme activity
  - inhibitor, inhibitor conc
  - buffer composition, T, duration, pre-washing and washing
- Seeding cell density
- Additives
  - Beads type and concentration
  - Beads conditioning
  - Inoculation ratio
- Cell transfer:
  - pump speed/pressure, duration
  - Cell attachment
  - agitation, duration

Method/material: growth in bioreactor
- pH
- pO2
- T
- pCO2
- Pressure
- Headspace aeration rate
- Sparger aeration (via pO2 regulation)
- Agitation speed: impeller
- Duration
- Scale-up
- Bioreactor design (engineering)
- Additives

Environment/Raw material
- 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)

Measure (in-process testing)
- Cell count/viability
- Metabolites
- pO2, pH, pCO2, T

CQA:
- HCP
- DNA
- Total protein
- Virus integrity
KPA:
- Cell density end growth
- Antigenic liter (SRD)
Environment/ Raw material

- Raw material control
- Media preparation
- Media filtration
- Media preheating
- Medium concentration
- Medium composition
- Feed (time, volume, conc)

Flexibility of the Environment

- Gas environnement
- Media storage/shelf-life (T, duration, light protection)

CQA:
- HCP
- DNA
- Total protein,
- Virus integrity

KPA:
- Cell density and growth
- Antigenic titer (SRD)
Method/material: growth in bioreactor

- pH
- pO₂
- pCO₂
- T
- Pressure
- Headspace aeration rate
- Sparger aeration (via pO₂ regulation)
- Agitation speed: impeller
- Duration
- Scale-up
- Bioreactor design (engineering)
- Additives

CQA:
- HCP
- DNA
- Total protein
- Virus integrity

KPA:
- Cell density end growth
- Antigenic titer (SRD)
Method: bead to bead

- Cell detachment
  - enzyme type, enzyme activity
  - enz. inhibitor, inhibitor conc.
  - buffer composition, T, duration, pre-washing and washing

- Seeding cell density
- Additives

- Beads type and concentration
- Beads conditionning

- Inoc ratio

- Cell transfer:
  - pump speed/pressure, duration

- Cell attachment
  - agitation, duration

CQA:
- HCP
- DNA
- Total protein,
- Virus integrity

KPA:
- Cell density and growth
- Antigenic titer (SRD)
Measure (in-process testing)

Cell count/viability

Metabolites

pO2, pH, pCO2, T

CQA:
HCP
DNA
Total protein,
Virus integrity
KPA:
Cell density and growth
Antigenic titer (SRD)
Infection – viral production

Method: infection
- Cell characteristics (passages)
- Seed characteristic
- Seed thawing
- MOI
- Seed activation (concentration of protease and duration of action)
- Additives
- Cell: density and physiological state

Environment/raw material
- Raw material control
- Media preparation
- Media storage/shelf-life (T, duration, light protection)
- Medium concentration
- Medium composition
- Feed (time, volume, conc) or/and perfusion (time, flow rate, conc)

Method/material viral replication
- pH
- pO2
- T
- pCO2
- Pressure
- Headspace aeration rate
- Sparger aeration (via pO2 regulation)
- Agitation speed: impeller
- Duration
- Scale-up
- Bioreactor design (engineering)
- Stabilizer

Measure (in-process testing)
- Cell count/viability
- Metabolites
- pO2, pH, pCO2, T

CQA:
- HCP
- DNA
- Total protein,
- Virus integrity
KPA:
- Cell density end growth
- Antigenic titer (SRD)
Method: infection

Cell characteristics (passages)
Seed characteristic
Seed thawing
MOI
Seed activation (concentration of protease and duration of action)
Additives
T
Cell: density and physiological state

CQA:
- HCP
- DNA
- Total protein
- Virus integrity

KPA:
- Cell density and growth
- Antigenic titer (SRD)
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)

Risk score
Ranking of effects
What might go wrong?
How severe are the consequences?
What is the likelihood the failure occurs?
Can we detect the failure?

Cross functional involvement is important
R&D, Process dev., Manufacturing, Sourcing, QA, QC etc
QbD workflow:
Risk analysis, FMEA

Impact

<table>
<thead>
<tr>
<th>Probability</th>
<th>Very Low</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>Very High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very High</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>High</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Medium</td>
<td>3</td>
<td>6</td>
<td>12</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Low</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Very Low</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>
## QbD workflow: Risk analysis, FMEA

### Define what each level represent

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

S - Severity of each failure (1-10)  
O - Likelihood of occurrence (1-10)  
D - Likelihood of detection (1-10)  
Risk Score = \( S \times O \times D \)
## QbD workflow: Risk analysis, FMEA score card

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

Actions on high scored risks

Identification of critical unit steps/critical unit step parameters for further examination, i.e. DoE
## Failure Mode and Effects Analysis (Process FMEA) Cell Bank

### FMEA Cell Bank

<table>
<thead>
<tr>
<th>Item / Process Step</th>
<th>Potential Failure Model(s)</th>
<th>Potential Effect(s) of Failure</th>
<th>Potential Mechanism(s) of Failure</th>
<th>Potential Cause(s) of Failure Mechanism</th>
<th>Sources of Variability - (think Fishbone)</th>
<th>Current Process Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freezing medium</td>
<td>DMSO low</td>
<td>low Viability</td>
<td>6</td>
<td>operator failure</td>
<td>stress, mis calculation</td>
<td>viability check 1</td>
</tr>
<tr>
<td></td>
<td>DMSO high</td>
<td>low Viability</td>
<td>6</td>
<td>operator failure</td>
<td>stress, mis calculation</td>
<td>viability check 1</td>
</tr>
<tr>
<td></td>
<td>serum not included</td>
<td>low Viability</td>
<td>6</td>
<td>operator failure</td>
<td>stress, mis calculation</td>
<td>viability check 1</td>
</tr>
<tr>
<td></td>
<td>wrong medium</td>
<td>low Viability</td>
<td>6</td>
<td>operator failure</td>
<td>stress, mis calculation</td>
<td>viability check 1</td>
</tr>
<tr>
<td></td>
<td>contamination</td>
<td>bank unusable</td>
<td>8</td>
<td>raw material contaminated</td>
<td>manufacturer</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>contamination</td>
<td>bank unusable</td>
<td>8</td>
<td>lab bench failure filter</td>
<td>service</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>contamination unknown</td>
<td>bank unusable</td>
<td>8</td>
<td>operator failure</td>
<td>stress</td>
<td>no</td>
</tr>
<tr>
<td>Storage</td>
<td>N2 min limit reached</td>
<td>bank unusable</td>
<td>8</td>
<td>operator failure</td>
<td>routines, communication</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>electrical failure (freezer)</td>
<td>bank unusable</td>
<td>8</td>
<td>equipment malfunction</td>
<td>power outage</td>
<td>alarm and service 1</td>
</tr>
<tr>
<td>Thawing protocol</td>
<td>too fast</td>
<td>low Viability</td>
<td>4</td>
<td>operator failure</td>
<td>water temperature high</td>
<td>viability check 1</td>
</tr>
<tr>
<td></td>
<td>too slow</td>
<td>low Viability</td>
<td>4</td>
<td>operator failure</td>
<td>water temperature low</td>
<td>viability check 1</td>
</tr>
<tr>
<td></td>
<td>wrong size of T-flask</td>
<td>low Viability</td>
<td>4</td>
<td>operator failure</td>
<td>stress</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>no DMSO removal</td>
<td>low Viability</td>
<td>4</td>
<td>operator failure</td>
<td>SOP</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>contaminated cell bank</td>
<td>bank unusable</td>
<td>8</td>
<td>operator failure</td>
<td>insufficient aseptic handling</td>
<td>sterility test 1</td>
</tr>
<tr>
<td></td>
<td>contaminated cell bank mycoplasma</td>
<td>bank unusable</td>
<td>8</td>
<td>operator failure</td>
<td>stress</td>
<td>mycoplasma test 1</td>
</tr>
<tr>
<td></td>
<td>Freezing</td>
<td>low cell number due to cell death</td>
<td>6</td>
<td>operator failure</td>
<td>xg/rpm</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>centrifugation speed high</td>
<td>low centrifugation recovery</td>
<td>2</td>
<td>operator failure</td>
<td>xg/rpm</td>
<td>pellet size / opaque supernatant 1</td>
</tr>
<tr>
<td></td>
<td>centrifugation speed low</td>
<td>low centrifugation recovery</td>
<td>2</td>
<td>operator failure</td>
<td>xg/rpm</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>cell density low</td>
<td>low Viability</td>
<td>5</td>
<td>cell counter malfunction</td>
<td>no service, insufficient maintenance</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>cell density low</td>
<td>low Viability</td>
<td>5</td>
<td>operator failure</td>
<td>calculation error</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>inhomogeneous cell suspension</td>
<td>variable quality</td>
<td>7</td>
<td>operator failure</td>
<td>insufficient mixing</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>uncontrolled freezing conditions</td>
<td>low Viability/cell death</td>
<td>6</td>
<td>operator failure</td>
<td>forget to add isopropanol</td>
<td>min limit mark 1</td>
</tr>
<tr>
<td></td>
<td>contamination</td>
<td>bank unusable</td>
<td>8</td>
<td>operator failure</td>
<td>insufficient aseptic handling</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>cells in plateau phase</td>
<td>low viability</td>
<td>5</td>
<td>operator failure</td>
<td>cell density too high</td>
<td>cell counting 1</td>
</tr>
<tr>
<td></td>
<td>passage number too high</td>
<td>thawing of new vial more often</td>
<td>3</td>
<td>operator failure</td>
<td>lack of experience</td>
<td>passage number check 1</td>
</tr>
<tr>
<td></td>
<td>Purchase of new cell line</td>
<td>contaminated ampoule</td>
<td>8</td>
<td>manufacturer failure</td>
<td>manufacturer routines</td>
<td>sterility test 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>variation in quality / increased work load</td>
<td>3</td>
<td>operator failure</td>
<td>lack of experience</td>
<td>no</td>
</tr>
<tr>
<td>Size</td>
<td>low number of vials</td>
<td>variation in quality / increased work load</td>
<td>3</td>
<td>operator failure</td>
<td>QbD Vaccines 2013-03-18_TinaG</td>
<td>8</td>
</tr>
</tbody>
</table>
Pareto plot Bioreactor conditions
Design of Experiments
QbD workflow: Design of Experiments (DoE)

DoE structured approach

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

Needed explanation level

One factor at a Time

Information %

Number of experiments

DoE structured approach
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

K. Johnson et al.
### Examples of DoE studies in cell culture PD

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

<table>
<thead>
<tr>
<th></th>
<th>Plates</th>
<th>Mini bioreactors</th>
<th>Standard bioreactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cultures</td>
<td>Very high</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>Culture volume</td>
<td>Low</td>
<td>Low</td>
<td>Larger</td>
</tr>
<tr>
<td>Factor screening</td>
<td>Broad</td>
<td>Broad</td>
<td>OFAT&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sensor options</td>
<td>No</td>
<td>Limited</td>
<td>Yes</td>
</tr>
<tr>
<td>Automation</td>
<td>Manual Robot</td>
<td>Manual Robot</td>
<td>Bioreactor system</td>
</tr>
</tbody>
</table>

<sup>1</sup> OFAT = one factor at a time
Analytics for Influenza Virus Vaccines
Analytics in DoE

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

- Virus + detergent
- 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

High titer

Low titer
**Biacore™ assay setup**

- **Anti-HA-Ab binding to:**
- **The virus in the sample**
- **Influenza HA proteins immobilized on the surface**

**Concentration of virus**
- LOW

**Response from surface**
- HIGH
- LOW

Quantification of a 3 strain seasonal influenza vaccine

Biacore™ assay

Final TBV

Hemagglutinin

Assay time: 100 samples, incl. controls & standards

Hands on time
Total analysis time
LOQ
Precision (# samples with CV<5%)

1 multiplexed assay
1-2h
15-16h
1 µg/ml
95%

3 separate assays
6-8 h
20-22 h total
12 µg/ml
18%
## Summary Biacore vs SRID

<table>
<thead>
<tr>
<th></th>
<th>Biacore™</th>
<th>SRID</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dynamic range</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg HA/mL)</td>
<td>0.5-10</td>
<td>5-20</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOD (µg HA/mL)</td>
<td>0.5</td>
<td>5</td>
</tr>
<tr>
<td>LOQ (µg HA/mL)</td>
<td>1.0</td>
<td>10</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%) samples CV &lt; 5%</td>
<td>97</td>
<td>18</td>
</tr>
<tr>
<td><strong>Recovery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>95-105</td>
<td>90-110</td>
</tr>
<tr>
<td><strong>Time for 100 samples</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hands-on (hours)</td>
<td>1 - 2</td>
<td>6 - 8</td>
</tr>
<tr>
<td>Total (hours)</td>
<td>15 - 16</td>
<td>20 - 22</td>
</tr>
</tbody>
</table>
Ishikawa diagram

Inoculum Preparation
- Thawing
- Split
- Medium
- Equipment
- Morphology
- Detachment

Cell Bank
- Fry's medium
- Storage
- Size
- Viability
- Quality testing
- Thawing protocol
- Freezing

Bioreactor Conditions
- pH
- DO
- CO2
- O2
- Temperature
- Equilibration
- Medium and supplements
- Metabolites
- Agitation
- Data log
- Microcarrier choice
- Cell counting/viability
- Sampling procedure

Bioreactor Equipment
- System check
- Probe calibration
- Bioreactor type
- System assembly
- Sterilization/integrity
- Pump
- Addition bottles
- Service
- Gas