# Virus safety in plasma-derived therapeutics:

# A Merck perspective

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**Louis Wong**

**Associate director, Plasma Initiative, Asia-Pacific**

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# Content

- Regulatory Expectations for Plasma-Derived Therapeutics
- Multi-Layer Strategy for Virus Safety

Prevention - Raw Materials and Chemicals

Removal – Clearance Technologies

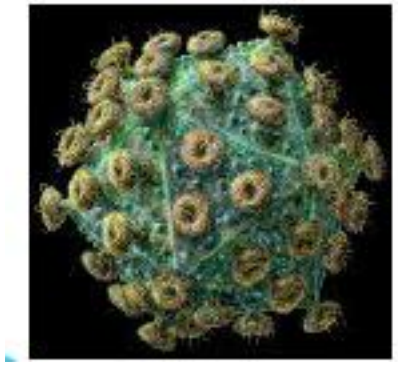
Detect – In-Process and Final Product Testing

- BioReliance Testing Services

# Virus Contamination in Plasma – A Reality

**Plasma Product Contamination**

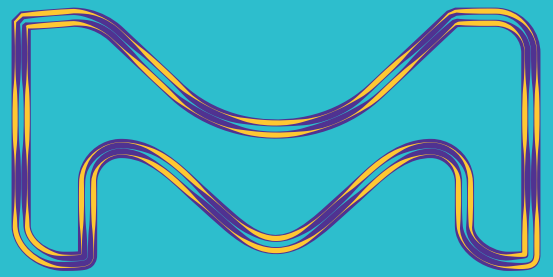
- 1980s: HIV contaminated Coagulation Factor
- 1994: Hepatitis C contaminated IgG
- 1995: vCJD contamination
- Other threats more recently… Hepatitis E, West Nile Virus, Ebola, Zika…



# Virus Safety in Plasma : Part of a Total Risk Mitigation Program

- ✓ Regular, qualified donors
- ✓ Donation control (serological and NAT tests)
- ✓ Inventory Hold, look back procedure
- ✓ Serological and NAT Tests on plasma pool
- ✓ **cGMP**
- ✓ **Pathogen inactivation/removal steps**
- ✓ **Batch Release**
- ✓ Post-marketing surveillance, pharmacovigilance

# Regulatory expectations for human plasma protein products



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# Regulatory Expectations for Human Plasma Protein Products

- Guideline on plasma-derived medicinal products. EMA/CHMP/BWP/706271/2010 (2011)
- ✓ Incorporate at least 2 orthogonal, effective virus inactivation/removal steps with at least 4 log virus reduction in each step is desirable
- ✓ Virus Inactivation methods/Virus removing filters

| Virus Group Choices | Types | Details |
|---|---|---|
| Enveloped Viruses | HIV 1<br>Model for Hepatitis C | Pestivirus BVDV could be considered a "worst-case" for HCV. |
| **Enveloped DNA Viruses** | Herpes Virus<br>Hepatitis B (duck model) | A validation study should be performed with an appropriate enveloped DNA virus, e.g. a herpesvirus such as pseudorabies. |
| **Non-enveloped Viruses** | Hepatitis A<br>B19V | HAV and B19V should be used for validation studies for coagulation factors (History of transmission). |
| **Model viruses for virus reduction filtration (nanofiltration)** | HIV<br>BVDV | For small pore size filters designed for removal of small non-enveloped viruses, HIV and BVDV should still be part of the virus panel, but robustness studies may focus on small non enveloped viruses. For medium pore size filters, BVDV is appropriate for robustness studies. |

# Virus inactivation/removal capacity
## *Points to consider for specific product classes*
EMA/CHMP/BWP/706271/2010 (2011) / Section 8.3

| Product Class | Details |
|---|---|
| Coagulation Factors | Non-enveloped viruses such as hepatitis A and B19V have been transmitted by this class of products. For Factor IX products, steps should be included in the process that are effective for HAV and B19V. |
| Immunoglobulins | Immunoglobulin products have a good safety record for the known non-enveloped viruses due in part to the contribution from neutralising antibodies in the product. |
| Albumin | Albumin manufactured by an established fractionation process that includes the terminal pasteurisation specified in the European Pharmacopoeia monograph, has an excellent virus safety record. |
| S/D Plasma | SD plasma has good safety measures for enveloped viruses and safety measures are in place for HAV and B19 (Ph. Eur. monograph Human Plasma (Pooled and Treated for Virus Inactivation). |

# Manufacturing Process Validation Activities by Drug Development Phase

| Process Validation Activities | Clinical Phases | Supporting Activities |
|---|---|---|
| | | ---- Initiate Process and Assay Development<br>Initiate Preliminary Process Characterization<br>Establish Preliminary Reference Standard |
| Perform Preliminary Clearance of Process Impurities | | |
| | | ---- Initiate Clinical Manufacturing |
| **Initiate Virus Clearance Studies** | 1 | |
| ---- | | ---- Initiate Stability Studies |
| | | ***Submit IND Application*** |
| | | Conduct Continuing Process & Assay Development |
| Initiate Manufacturing Process Characterization ----<br>Studies (test limits) | 2 | Qualify or Validate Assays |
| Approve Manufacturing Process Validation Plan ---- | | ---- Establish Gene Therapy Product Characterization<br>---- Establish Manufacturing Process Definition |
| Develop/Approve Process Validation Protocols ---- | | ---- Conduct Pivotal Clinical Trails |
| **Conduct Additional Virus Clearance Studies** | | |

# Virus Safety: Multi-Layer Strategy
# A Merck Perspective

1


## Prevent

**Virus Safety of Raw Materials**

2

## Detect

**Testing of In-Process and Final Product**

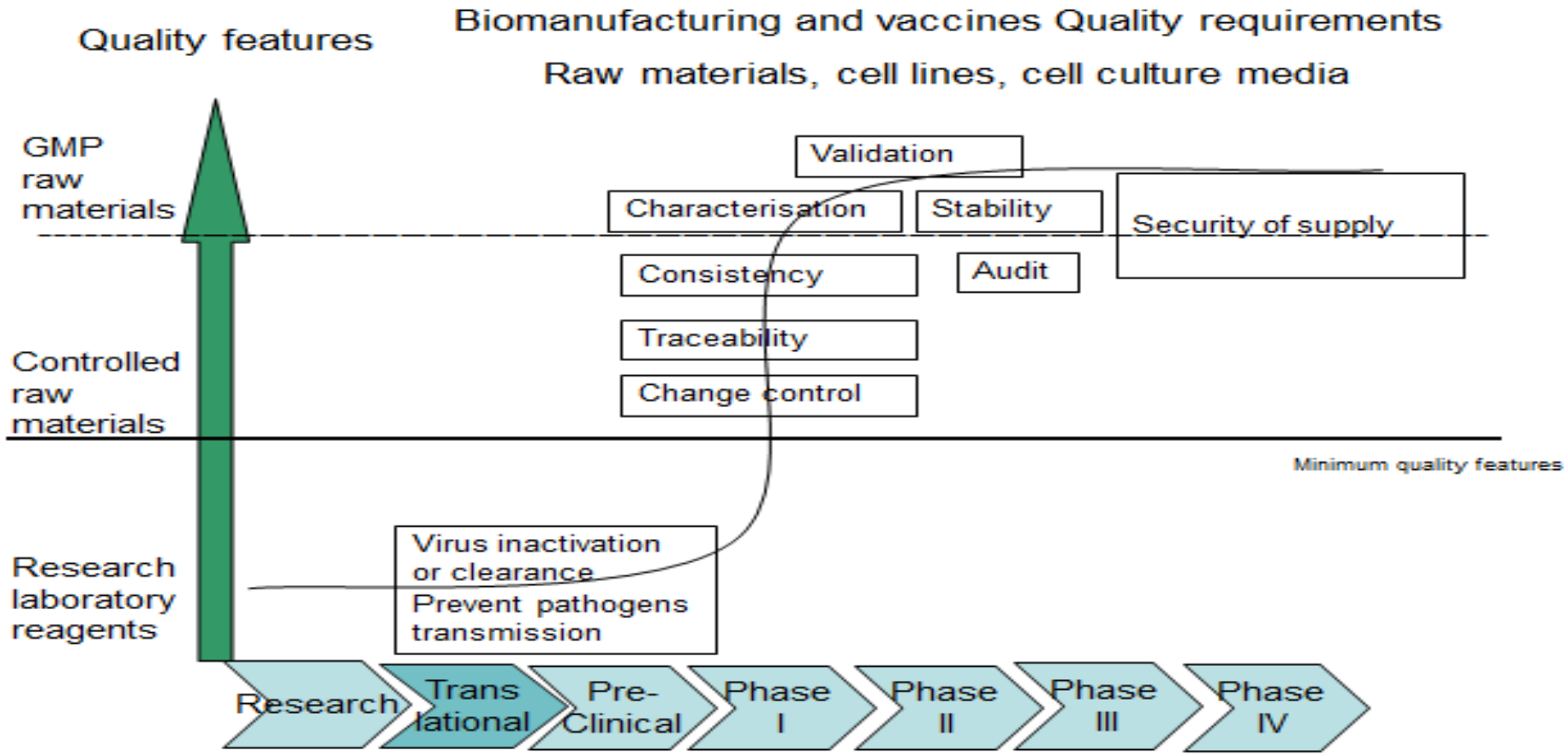
3

## Remove

**Virus Clearance Technologies**

# Overcoming challenges to transition your raw materials
## **Start with the end in mind**

- *Quality standards differ for raw materials*
- *Quality requirements increase for drugs approaching commercial launch*

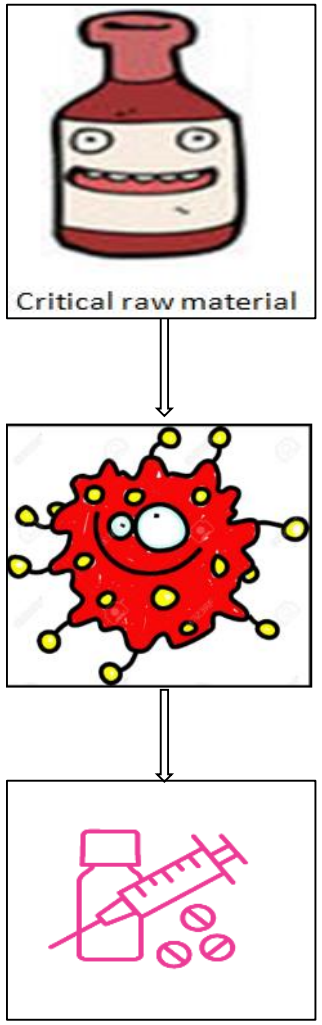


# Overcoming challenges to transition your raw materials

## Comparison of approaches

### Traditional development

- ✓ Focus on end product safety and performance
- ✓ Fixed process – do not change make any changes after Research
- ✓ Fixed specifications
- ✓ Work with suppliers starting phase II




### Enhanced development

- ✓ Systematic – understand material features and process to meet critical to quality attributes
- ✓ Supply Chain Risk Management to ensure uninterrupted drug supply
- ✓ Work with suppliers who can support you in transitioning to Clinical and comply with Regulatory requirements

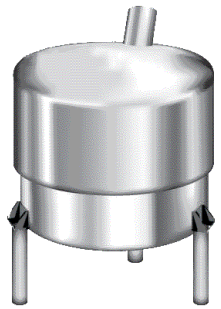
# The Merck EMPROVE® Concept

The EMPROVE concept brings a package of benefits to the plasma fractionation customer that include:

- Strong process safety and reliability – High quality raw materials with GMP production;
- Strong regulatory support – EMPROVE dossier in CTD format allows the customer to save time and money;
- Full and permanent access to main documents in our website;
- Top level Quality Control procedures, covering the manufacturing and the supply chain steps.

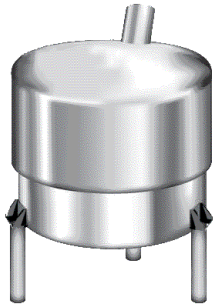
Quality, Safety and Stability are the key words in the EMPROVE® brand products for blood plasma fractionation

# General Fractionation Process Steps with EMPROVE® Chemicals



Cryoprecipitation

Multiple steps



Precipitation

Centrifugation/
Depth Filtration

Dissolution of
Fraction II/III paste

Multiple steps

SD Inactivation
/Low pH

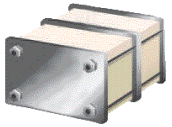
<u>Stabilizers</u>
EMPROVE® exp
Glycine
Sodium Chloride

<u>Precipitation</u>
EMPROVE® exp
PEG4000
Sodium Capylate

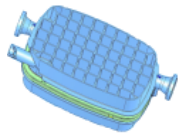
<u>Buffers</u>
Acetates/Phosphates
Sodium Hydroxide

<u>SD/Low pH</u>
EMPROVE® exp
TnBP, Triton®X-100,
Tween® 80
Acetic Acid, Sodium
acetate trihydrate,
Caprylic Acid

Anion Exchange
Chromatography



Concentration/
Diafiltration

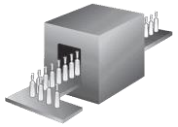


Nanofiltration

Formulation

<u>Bulking Agents/Stabilizers</u>
EMPROVE® exp Sucrose,
Sorbitol, Maltose,
L-Alanine, Glycin
N-Acetyl-DL-tryptophan

Final filtration



Filling

Lyophilisation

Liquid Fill

# Virus Safety: A Multi-Layer Strategy


## Prevent

**Virus Safety of Raw Materials**


## Detect

**Testing of In-Process and Final Product**


## Remove

**Virus Clearance Technologies**

# Types of Process Steps

EMA/CHMP/BWP/706271/2010 Guideline on plasma-derived medicinal products

> "For all plasma-derived medicinal products, it is an objective to incorporate effective steps for inactivation/removal of a wide range of viruses of different physico-chemical characteristics."

**Inactivation**

- Heat Treatment
  - Pasteurization (60°C, 10 hours, Albumin)
  - Lyophilization / dry heat
- Solvent/Detergent (TnBP, Triton X-100, Tween 80)
- Low/High pH (Caprylic acid, Acetic acid)
  - Column Elution/Sanitization

**Partitioning**

- Precipitation
  - EtOH
  - PEG
  - Caprylic Acid
- Chromatography
  - Ion Exchange
  - Affinity
  - Gel Filtration
- NanoFiltration

# Viral Reduction Capacities by Contributive Steps

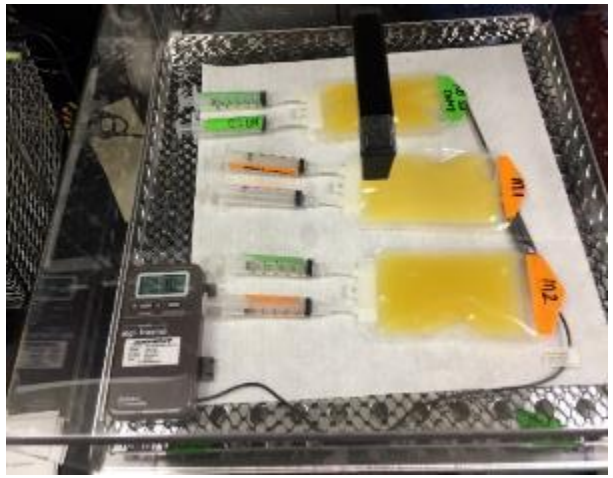
| Spiked viruses | | ENVELOPED VIRUSES | | | | NON-ENVELOPED VIRUSES | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | HIV-1 | Sindbis | BVDV | PRV | SV-40 | EMCV | HAV | PPV |
| Model for | | HIV | HCV | | Enveloped DNA virus (HBV) | Highly resistant virus | HAV | | Parvovirus B19 |
| Specific steps | S/D treatment | e 4.4 | e 5.4 | NT | e 4.3 | N/A | N/A | N/A | N/A |
| | Nanofiltration through 20 nm filter | 5.6 ① | 5.6 ① | 5.6 | 5.6 ① | 5.4 ② | e 5.2 | NT | 4.3 |
| Contributive steps | Caprylic acid fractionation | e 4.0 | NT | 5.1 | e 5.0 | NT | e 5.6 | e 5.6 | 3.7 |
| | Anion-exchange chromatography | NT | NT | NT | NT | NT | 1.3 | NT | 3.8 |
| | Final product: LowpH incubation in the final container | 4.0 | NT | 3.2 | NT | NT | NT | NT | NT |
| | Final product: Virus-neutralising capacity by the antibodies | N/A | N/A | N/A | N/A | N/A | N/A | 3.3 ③ | N/A |
| Overall viral reduction capacity | | e 18.0 | e 11.0 | 13.9 | e 14.9 | 5.4 | e 12.1 | e 8.9 | 11.8 |

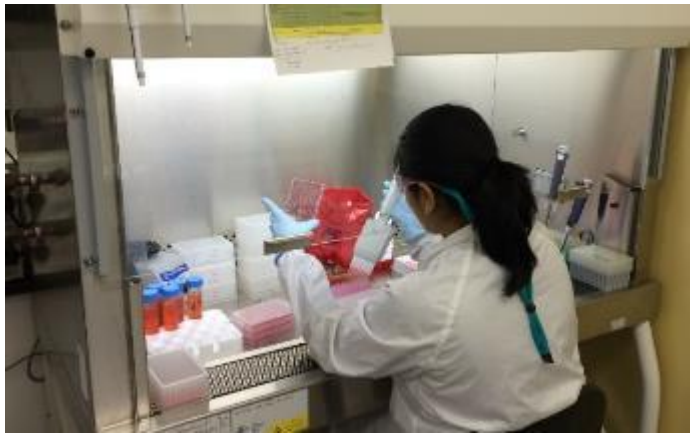
Source: Production of ClairYg, Christophe Segard, LFB

# Solvent/Detergent Viral Inactivation using Single-Use Technology

**Objectives:**

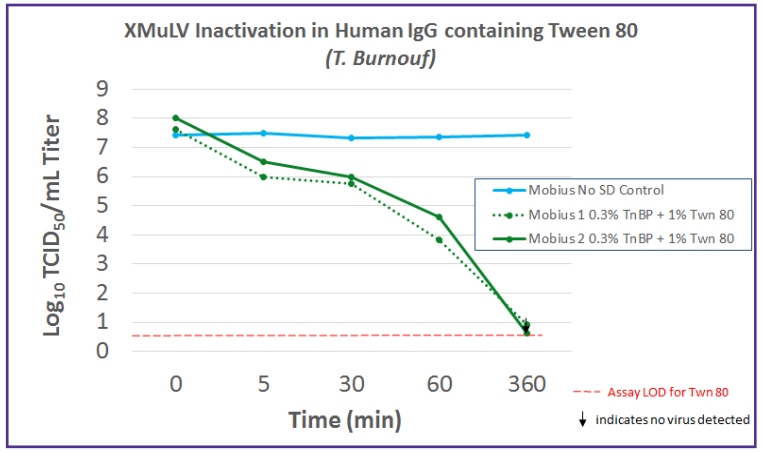
- Quantify Impact of S/D Treatment **on Chemical Compatibility**, **Extractables/Leachables** and **Mixing Efficiency** in Single-Use Bags
- Determine **Efficacy** of S/D Viral Inactivation in Single-Use Bags
- Investigate Impact on **Protein Activity**
- Create **Best Practices** for performing S/D Process Step in Single-Use Mixers

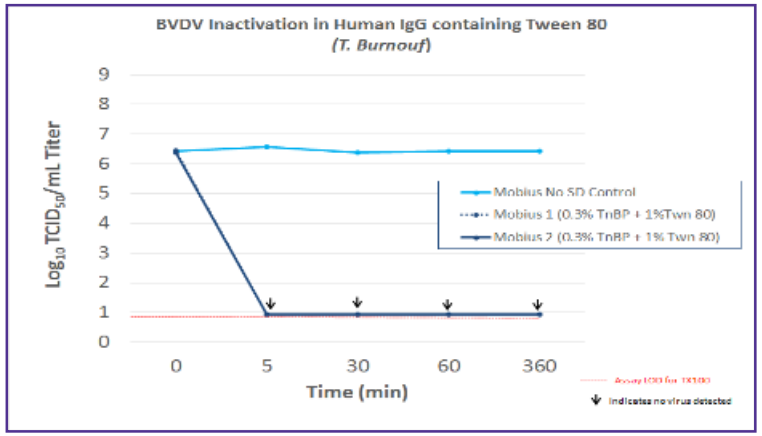




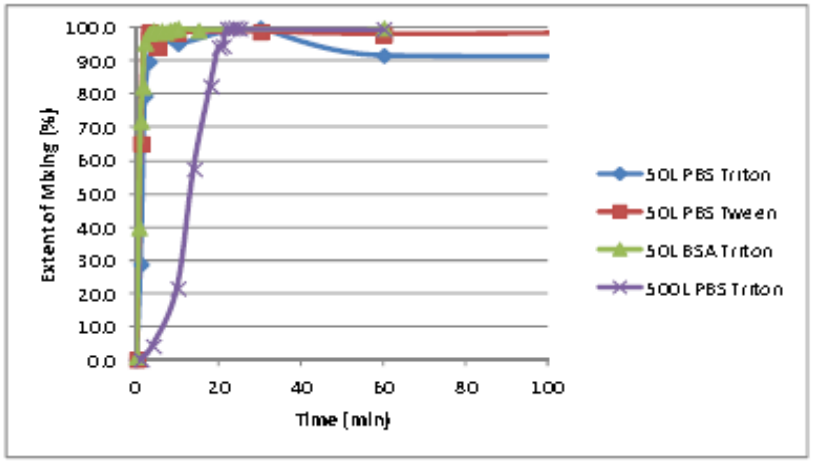
# Outcomes of Study

Virus Reduction on Human IgG performed in Mobius SU bags




Mixing Efficiency in Mobius SU-Mixer 50L and 500L



# Virus Removal by Nanofiltration

- Virus Filtration performed on 10 different Grifols plasma proteins/products
- Conclusion of Nanofiltration being extremely effective step in virus reduction
- Minimal influence of process parameters like pH, temperature, Conductivity, during NF

| Protein (or product) | | Enveloped viruses (≥40 nm) | | | | Non-enveloped viruses | |
|---|---|---|---|---|---|---|---|
| | | | | | | Small (<35 nm) | Very small (<25 nm) |
| | | PRV | HIV-1 | WNV | BVDV | HAV/EMCV | PPV |
| A | | ≥4.6 | ≥4.8 | ≥3.6 | ≥4.7 | ≥5.9 | 4.6 |
| B | | ≥6.1 | ≥5.6 | ≥4.5 | ≥4.5 | 5.2 | 4.4 |
| C | | ≥6.0 | ≥4.0 | ≥5.4 | ≥4.9 | 6.6 | 6.1 |
| D | D1 | ≥5.5 | ≥5.9 | ≥5.8 | ≥4.1 | ≥4.2 | 4.1 |
| | D2 | ≥5.2 | ≥6.9 | n.d. | ≥4.7 | ≥5.5 | 4.2 |
| E | E1 | ≥4.2 | ≥3.8 | ≥6.2 | ≥3.7 | ≥6.4 | 5.0 |
| | E2 | ≥5.4 | ≥5.4 | n.d. | ≥4.8 | ≥4.2 | 6.5 |
| F | F1 | ≥6.0 | ≥6.8 | ≥7.0 | ≥6.3 | ≥5.3 | 3.9 |
| | F2 | n.d. | ≥4.5 | ≥6.0 | 4.6 | ≥4.4 | 3.8 |
| G | | n.d. | ≥6.3 | n.d. | ≥5.1 | ≥5.6 | 4.6 |

BVDV: bovine viral diarrhea virus; EMCV: murine encephalomyocarditis virus; HAV: hepatitis A virus; HIV: human immunodeficiency virus; n.d.: not determined; PPV: porcine parvovirus; PRV: pseudorabies virus; WNV: West Nile virus.

Source: Robustness of Nanofiltration for increasing the viral safety margin of biological products
S Caballero et all, Grifols 2014

# Virus Clearance Validation




**Prevent**

**Virus Safety of Raw Materials**

**Detect**

**Testing of In-Process and Final Product**

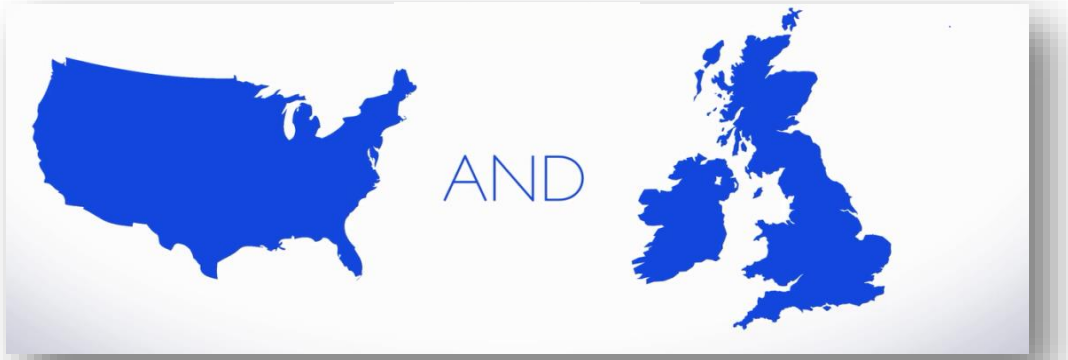
**Remove**

**Virus Clearance Technologies**

BioReliance® by SAFC

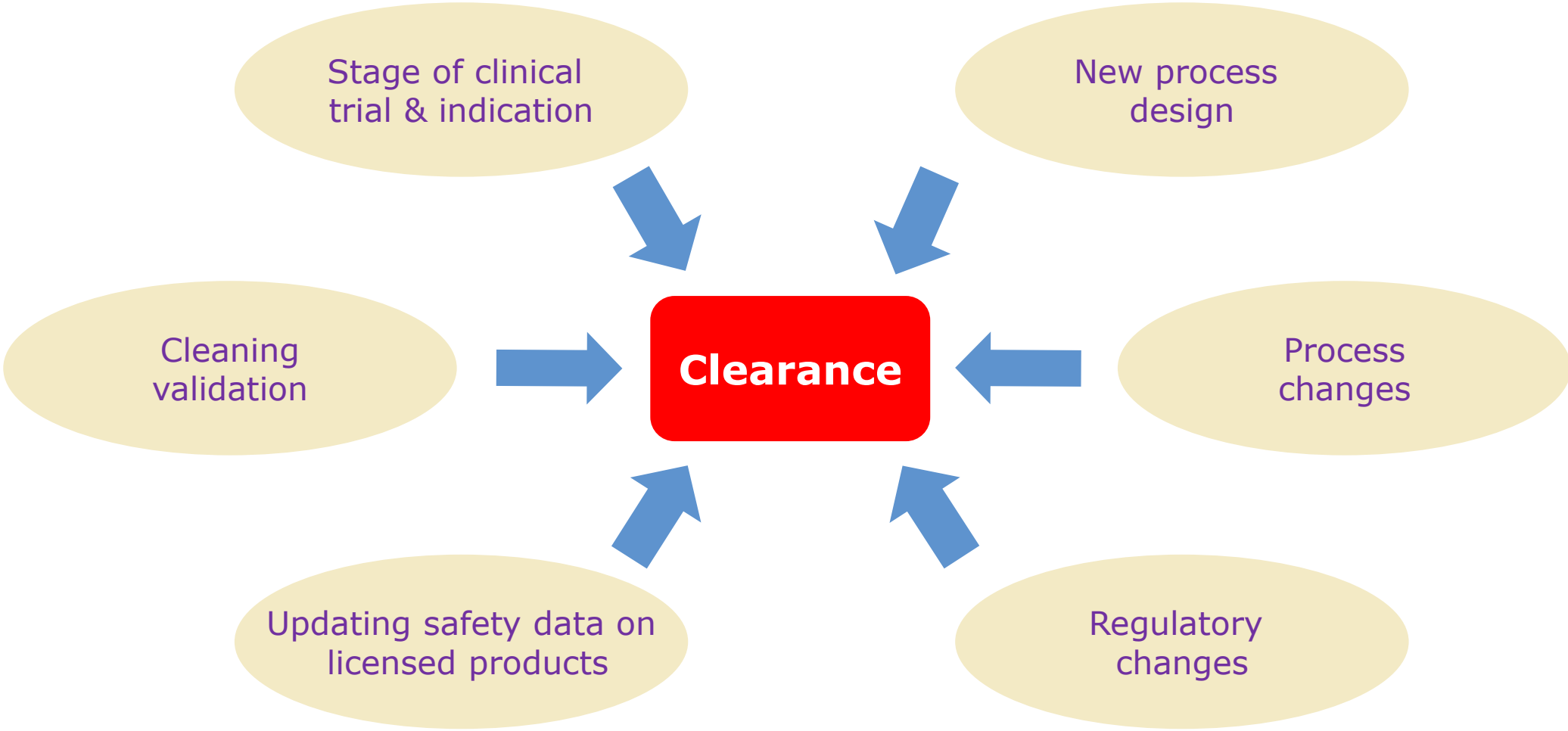
# BioReliance Clearance Services
## **Perform studies at 2 separate facilities**

- Dedicated facilities in both Rockville (MD), USA and Stirling, Scotland.
  - Rockville facility has 4 study suites and a BSL-3 lab.
  - Stirling facility has 5 study suites and a BSL-3 lab.
  - Both make use of state-of-the-art equipment Endpoint testing conducted in adjacent virus titration labs to increase the speed and efficiency of sample analysis.
- Trained and experienced personnel provide technical and regulatory support for all projects.
- Besides Viral Clearance and TSE Clearance studies, Mycoplasma, DNA and Bacterial Clearance studies can also be performed.



# Viral Clearance Study Design

# Viral Clearance Studies
## **When are they performed?**



# Viral Clearance Studies
## **Testing each process step**

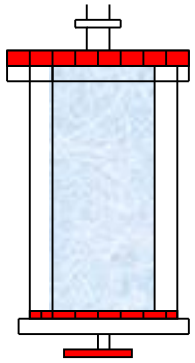
**Input Virus**

**Cleared Virus**

**Leftover Virus**

Spike virus into process intermediate. Must be appropriate to Load material (e.g., liquid vs. solid)

Perform scaled-down chromatography process step(s)



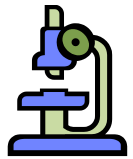
Collect fractions (e.g., flow-through, eluate, regenerate)

Solvent, Detergent, pH inactivation

Perform scaled-down filtration process step

Collect fractions

**Titrate for virus infectivity level (TCID50)***



$$\text{Reduction} = \frac{\text{[virus] load}}{\text{[virus] product}}$$

*qPCR for some removal steps

# Viral Clearance Studies
## **Generic timelines**

| Study Type | Wk 1 | Wk 2 | Wk 3 | Wk 4 | Wk 5 | Wk 6 | Wk 7 | Wk 8 | Wk 9 | Wk 9 | Wk 10 | Wk 11 | Wk 12 | Wk 13 | Wk 14 | Wk 15 | Wk 16 | Wk 17 | Wk 18 | Wk 19 | Wk 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Early Stage Clearance Study Timelines** | Design /SOW | | | | Pre Study | | | Spiking Study | | Assays | | | Draft report | | Final Report | | | | | | |
| **Late Stage Clearance Study Timelines** | Design /SOW | | | | Pre Study | | | Spiking Study | | | | Assays | | | Draft report | | Final Report | | | | |

**Total: 12-16 Weeks**

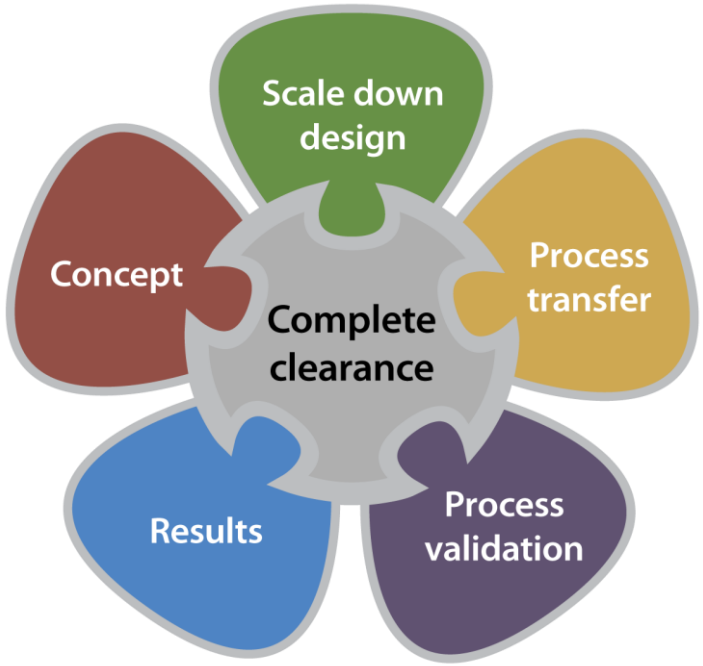
**Total: 16-20 Weeks**

**Performing a viral clearance study**

# Conclusion

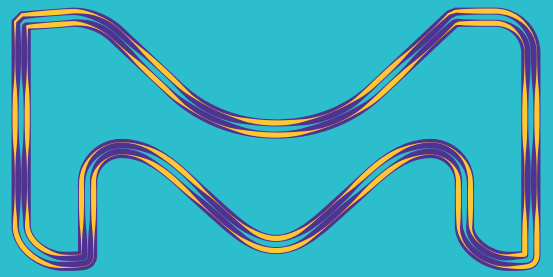
- Three-Pillar Approach for Total Virus Safety Considerations in Plasma
- Start with the end in mind
- Merck Key Competencies in Plasma:

Regulatory Experts, Virus Clearance Team, Process Validation Experience, Plasma Fractionation Process Knowledge and Leading Technologies



# Thank you!

contact:
Louis.Wong@Merckgroup.com



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