

Viral Safety Concepts for Gene and Cell Therapy Learned from Plasma-derived Products and Biotechnology

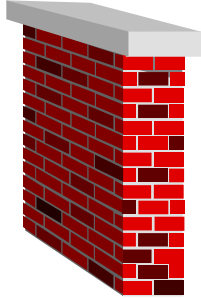


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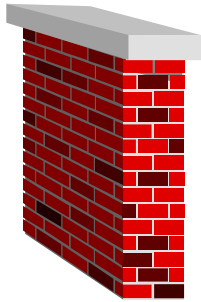
Three Viral Safety Strategies

1



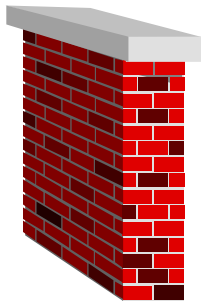
Selection of starting/raw materials

2



testing

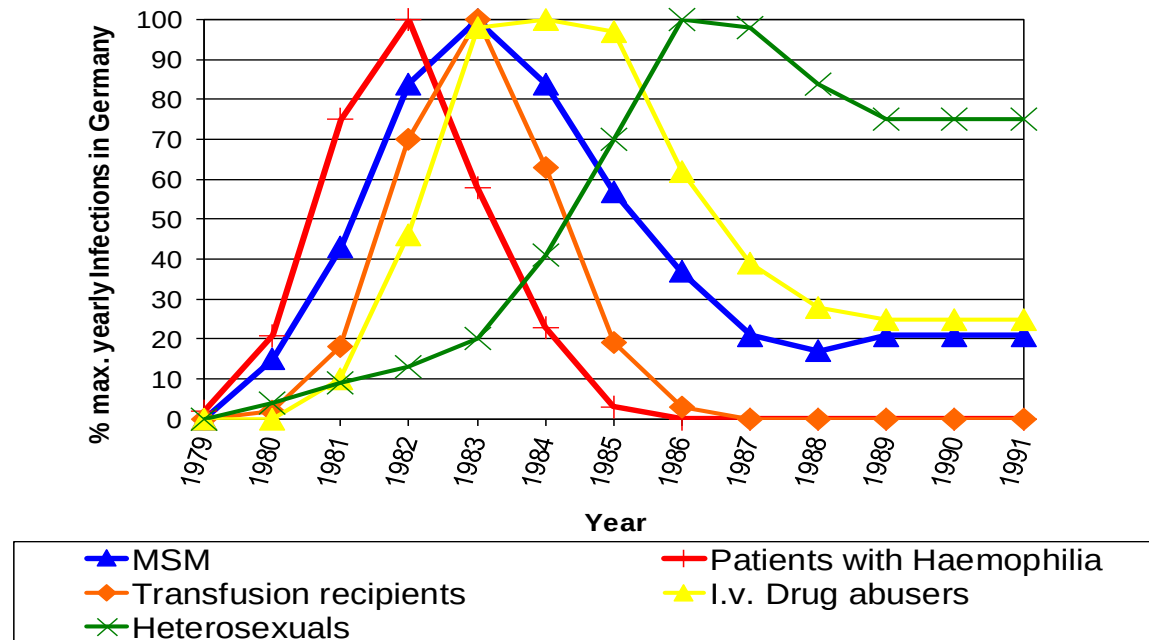
3



inactivation, removal

Lessons for Plasma Industry - The HIV Shock

- In the early years of enthusiasm about a fundamental improvement of life expectancy and quality, little attention was given to virus transmission. Substitution-associated hepatitis was taken as “transaminitis”
- The massive transmission of HIV by blood products was one of the worst disasters of modern medicine.
- The **fastest spread** of HIV from USA was **via plasma products**. Haemophilia patients in Germany became infected before HIV was identified and **1-2 yrs earlier than other risk groups**.



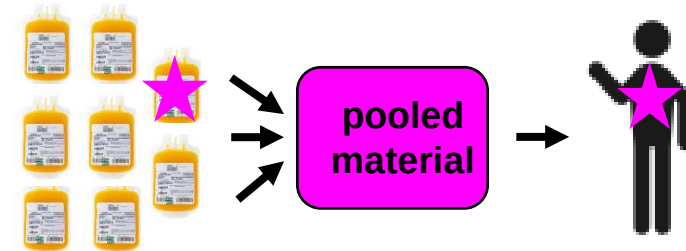
Risk of virus transmission by pooling and large scale production

Pooling of biological starting materials and large scale manufacture offers advantages with respect to consistent and economical production

However:

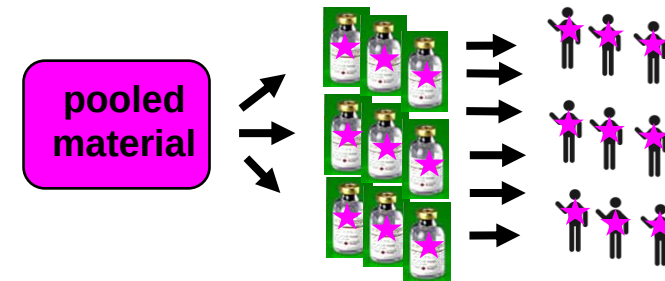
→ Pooling increases the probability of virus transmission to a single recipient

- Larger number of donors / donations
- Single donor contamination will contaminate the whole pool



→ Pooling increases the number of recipients getting contaminated blood/plasma

- contamination of one plasma pool will be distributed to many recipients

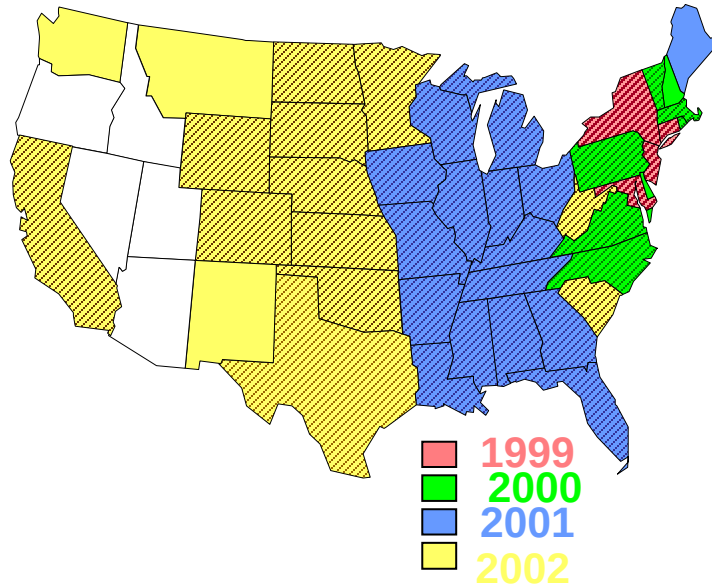


Lessons for Plasma Industry

- Risk from emerging pathogens
- Exclusion measures are not perfect
- Virus testing is not perfect
- Virus inactivation, originally designed to prevent Hepatitis B (and Non A Non B Hepatitis), worked against HIV-transmission.
- 1993/94: Transmission of HCV by iv.-IgG (no virus inactivation, unexpected)
- 1993/94: Transmission of HBV by Prothrombin-Complex
(failure of inactivation)
- Hepatitis A Virus: Transmission of a virus that we did not expect to be in blood
- Parvovirus B19: High viral loads may overload/overwhelm virus reduction steps.

It is important to have (multiple) virus inactivation/removal steps in place.

Success Story: West Nile Virus and Plasma Products



PPTA-Study 2002: Pools contain up to 5×10^2 genomes/ml WNV-DNA

PEI-Study 2006: Pools contain up to 5×10^2 genomes/ml WNV-DNA

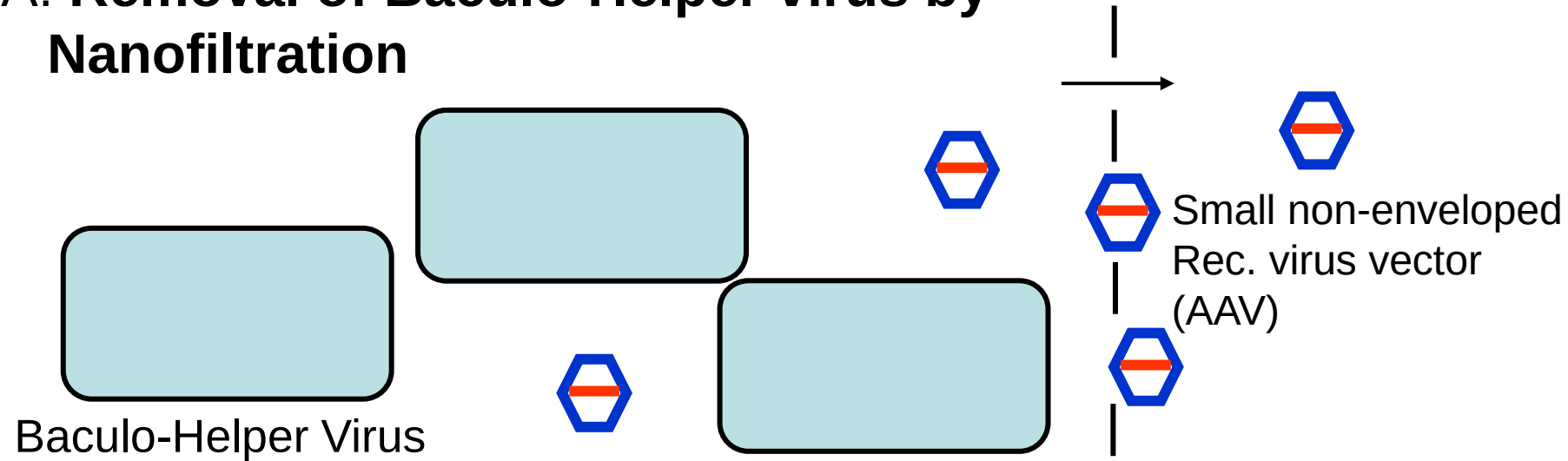
Double inactivation/removal of enveloped viruses has been effective against emerging enveloped viruses such as WNV.

Gene Therapy

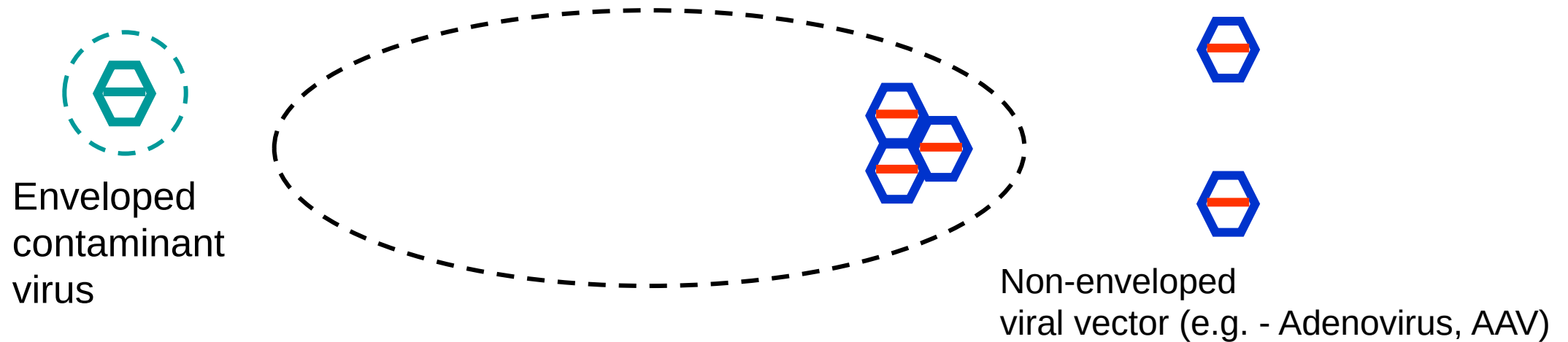
As for live virus vaccines, methods for virus inactivation/removal are not applicable for viral gene vectors

**Really?
Always?**

A: Removal of Baculo-Helper virus by Nanofiltration



B: (solvent)/detergent lysis of virus producing cells



Viral Inactivation at production of viral vectors (e.g. Adenoviruses, AAV)

Why:

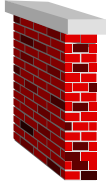
- Case: Contamination of SF9 cells with an insect Rhabdovirus [Ma et al., 2014. J Virol. 2014 Jun;88(12):6576-85] (Glybera).
- HEK 293 (human) cells are susceptible many to human pathogenic contaminants (cells have been optimized for viral replication).
- Additional safety barrier as usual for rec. proteins from cell cultures (e.g. CHO-derived proteins).

How:

- (Solvent)/Detergent lysis, Chromatography, additionally for for AAV-vectors: medium virus filters, low pH, chromatography , heat,

Cell Based Medicinal Products

Safety Concept: Focus on safe raw materials



Focus on selection of safe raw materials



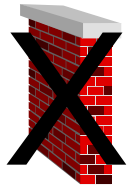
Often no cell banks to test

Often individual cell cultures

Often no end of production testing

(time restrictions, limitation of conventional assays for unknown viral contaminants)

→ new developments with NGS!



viral inactivation/removal methods are not applicable to growing/dividing cells

Lessons for Vaccine Industry

- Remember transmission of serum hepatitis (Hepatitis B) by yellow fever vaccine stabilized with human serum in late 1930s/1940s)
- Replacement of non-inactivated serum-excipient with pasteurized albumin
- Replacement of human serum with animal serum (FBS) ...species barrier.....
- Low level BVDV in bovine serum escaped testing and lead to contamination of bovine vaccine and dissemination of the disease (no species barrier)
- Use on virus inactivated Serum (>30kGy) in order to reduce cell culture contaminations

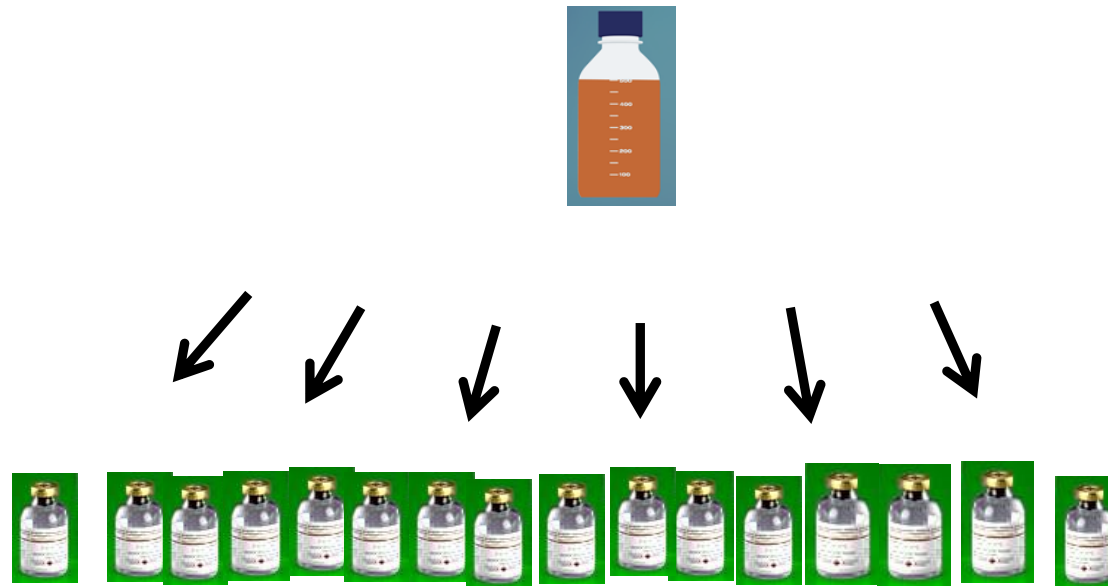
Trends in Cell Based Therapy

- Switching from bovine serum to (non-inactivated) human serum or human platelet lysates
- Use of pooled heterologous sera/ platelet lysates instead of autologous serum
- “... *human serum is more safe than FBS with respect to TSE...??*)

Case 1: One batch of non-inactivated human serum used as excipient for many containers of an autologous cell based medicinal product.

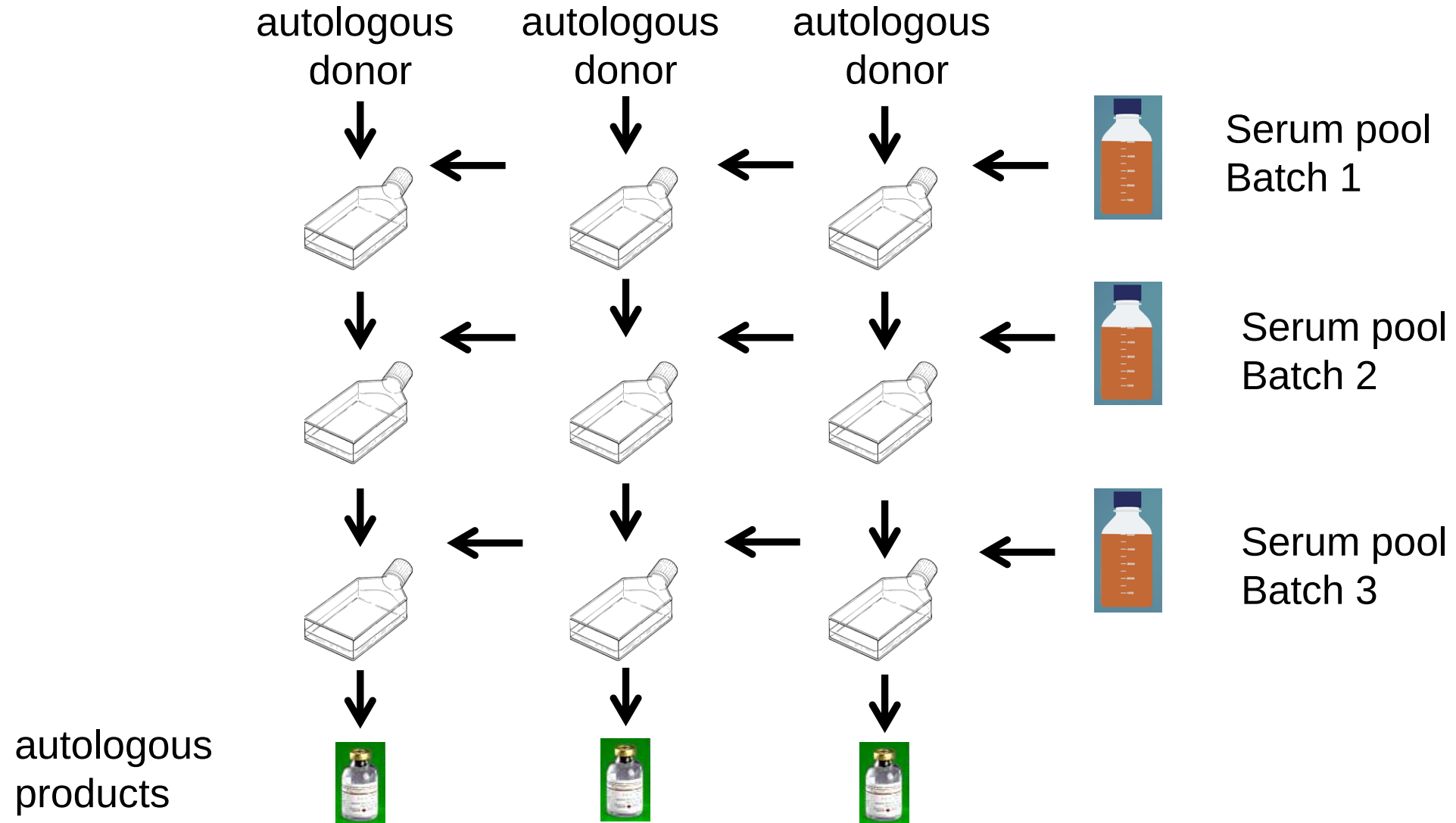
„....only 1ml serum per dose“,

„not willing to qualify multiple serum -excipient lots“



Advice: Replace human serum excipient with human serum albumin.

Case 2: Trend towards upscaling: Use of multiple serum batches for parallel processing of individual cell cultures .



Ph Eur. 5.2.12. Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

Use of serum or human platelet lysate (hPL) as raw material:

“...consideration is given to limit the number of donations which are pooled, unless sufficient methods for inactivation/removal of viruses are applied during production, where applicable.”

→ Small pool size or virus inactivation...

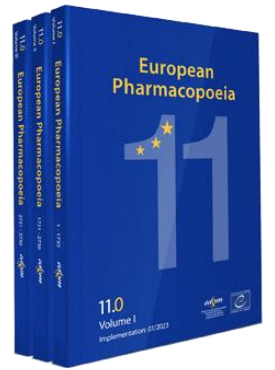
Without virus inactivation: PEI request to use only pools that contain no more than **16 donations**

→ This number should apply to the whole manufacturing process (entering in one final drug product) and not only to a single serum batch. 16 is enough for a homogenous pool.

Stühler and Blümel, Spezifische Aspekte zur Virussicherheit von Produktionshilfsstoffen für somatische Zelltherapie-Arzneimittel. Bundesgesundheitsbl 2015 · 58:1233–1238).

Henschler et al., Human platelet lysate current standards and future developments. TRANSFUSION;59:1407-1413, 2019.

It is preferred to implement virus inactivation. Low level contaminants may escape virus testing and amplify in cell culture



Reduction factor and Log Reduction Values (LRV)

A logarithmic reduction value (LRV) is the logarithmic value of the reduction factor from an virus inactivation experiment

The LRV **reflects the ratio** of virus before and after inactivation (e.g. LRV = 4 means a 10,000 fold inactivation).

The LRV **does not reflect the total amount of virus** that has been inactivated.

Overloading of virus reduction: Theoretical Example:

The inactivation experiment showed reduction of 10^6 infectious particles per ml towards 10^2 /ml.

This means that a total amount of $1,000,000 - 100 = 999,900$ infectious particles per ml were inactivated ...and not more...

In the case of overwhelming, a single 200ml donation containing 10^7 infectious particles per ml will be reduced towards $10,000,000 - 999,900 = 9,900,100$ infectious particles per ml. This means that $200 \times 9,900,100 = 1,980,020,000$ can survive the inactivation and will contaminate a serum pool.

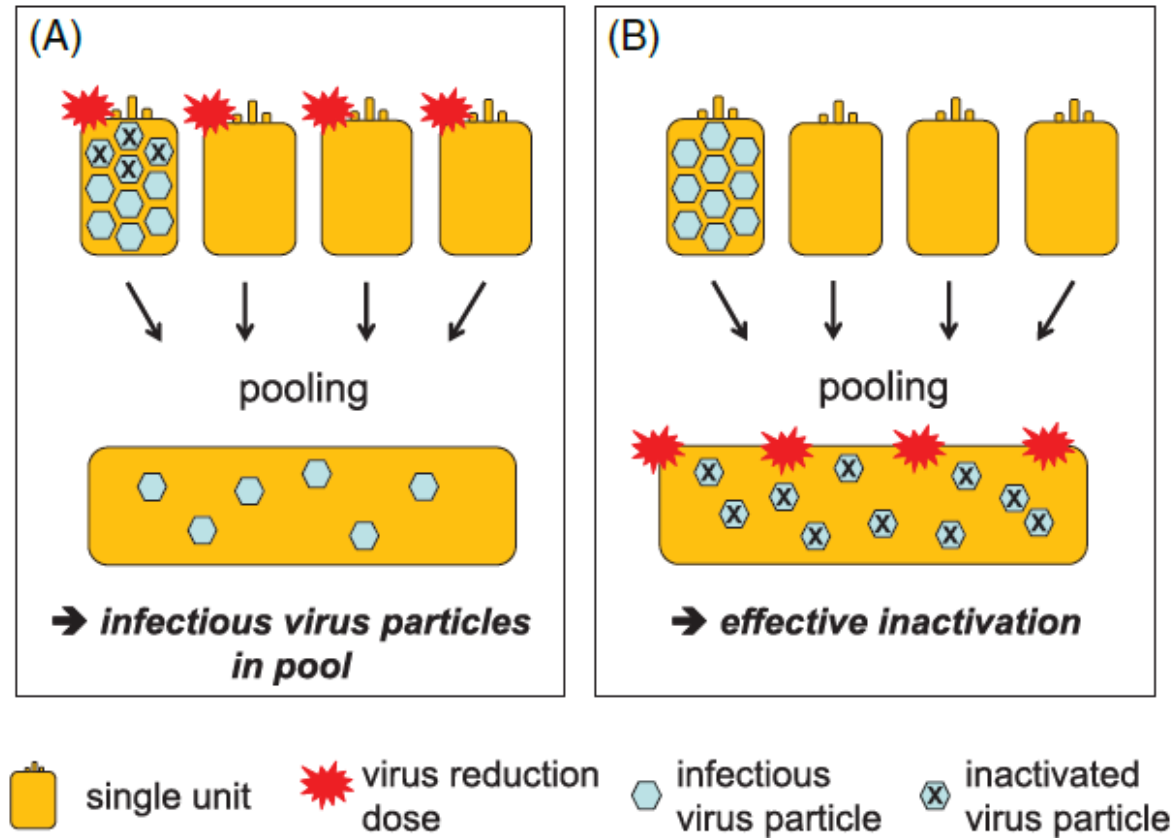
Overwhelming of Pathogen Reduction (real case)

Effect of *Plasmodium* inactivation in whole blood on the incidence of blood transfusion-transmitted malaria in endemic regions: the African Investigation of the Mirasol System (AIMS)

Jean-Pierre Allain, Alex K Owusu-Ofori, Sonny Michael Assennato, Susanne Marschner, Raymond P Goodrich, Shirley Owusu-Ofori

- Mirasol inactivation experiments showed **LRV of >6.4** for *Plasmodium falciparum*
- Transmission of parasites to **one of 28 recipients** treated with parasitaemic whole blood units subjected to pathogen reduction.
- Patient was transfused with two highly parasitaemic units: **7×10^8** copies per ml and developed increasing parasitaemia
- Patients exposed to fewer than 1×10^8 copies of parasites in treated whole blood did not develop transfusion-transmitted malaria.

Apply virus inactivation to pooled material rather than to single donations



Comparing viral inactivation methods applied before or after pooling of single units. In Scenario A, four doses of a virus inactivating agent (e.g., a chemical compound or an irradiation) are applied to individual platelet units; some virus particles are not inactivated and enter the pool. In Scenario B, the same overall inactivation dose as in Scenario A is applied to the pool, with effective inactivation of the homogeneously distributed virus particles

Virus inactivation for pooled human serum and platelet lysates

Gamma Irradiation: Viau, Eap, Chabrand, Lagrange, Delorme. Viral inactivation of human platelet lysate by gamma irradiation preserves its optimal efficiency in the expansion of human bone marrow mesenchymal stromal cells. *TRANSFUSION* 2019;59;1069–1079.

Intercept–solvent/detergent: Barro, Su, Nebie, Wu, Huang, Koh, Knutson, Burnouf. A double-virally-inactivated (Intercept–solvent/detergent) human platelet lysate for in vitro expansion of human mesenchymal stromal cells. *TRANSFUSION* 2019;59;2061-2073, doi:10.1111/trf.15251.

Nanofiltration: Barro, Nebie, Chen, Wu, Koh, Knutson, Watanabe Takahara, Burnouf. Nanofiltration of growth media supplemented with human platelet lysates for pathogen-safe xeno-free expansion of mesenchymal stromal cells, *CYTOTHERAPY* 2020;22;458-472 , doi: 10.1016/j.jcyt.2020.04.099.

Summary

Viral Inactivation/removal methods are applicable to some (non-enveloped) viral vectors and can eliminate non-recognized cell culture contaminants.

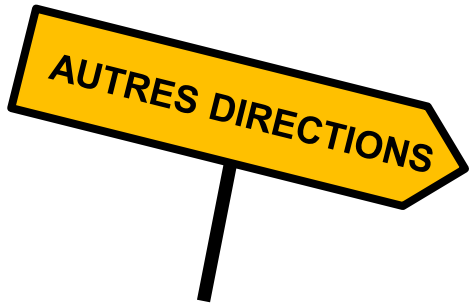
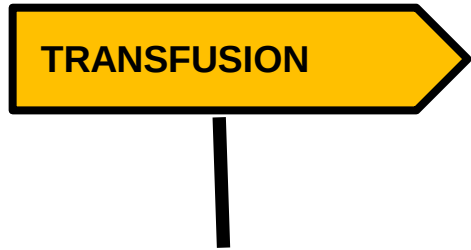
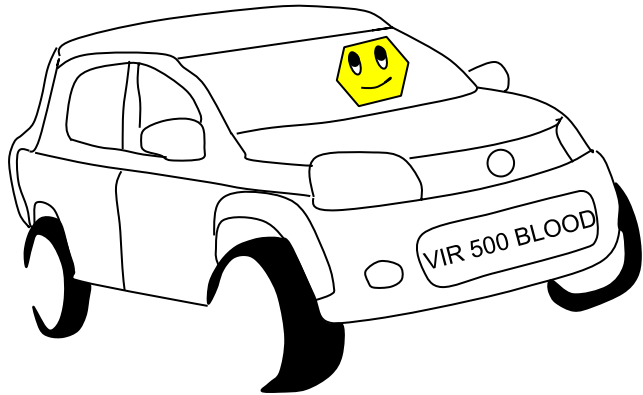
Safety of raw materials such as human serum or human platelet lysates (hPL) is crucial for cell based medicinal products

Avoid Serum/hPL in cell therapy ... if possible...
(or use autologous raw material)

Use virus-inactivated (pooled) serum or hPL.

Virus inactivation should be preferentially applied to the pooled material.

Thank you for the
Attention!



Internal

