Viral Clearance for Medical Devices

Oliver Schäfer*, Jürgen Raimann*, Nina Scherer+ and Horst Ruppach*

* Charles River Biopharmaceutical Services GmbH, Gottfried-Hagen-Str. 20, 51105 Cologne, Germany
+ Tutogen Medical GmbH, Research and Development, Industriestraße 6, D-91077 Neunkirchen am Brand, Germany

Introduction

The principles and the challenges of a virus clearance (VC) study for medical devices (1) are outlined. A solid tissue derived from an animal source is used as an example. The manufacturing process includes H_2O_2 treatment, which was analyzed for the capability to inactive the model viruses MuLV, PRV, Reo-2 and PPV.

Challenges

There following items make the design of a medical device VC study challenging:

- Source material not always sterile
- Harsh conditions, which leads to high toxicity.
- Source material often solid material.

Especially solid phase material requires careful attention to:

- the spiking process and
- the recovery of the virus from the solid material (virus extraction).

Spiking

- The tissue was cut into equal and representative pieces
- Virus was applied on tissue pieces by dropping small aliquots of virus on the tissue with subsequent drying.
- This step was repeated to achieve a reasonable viral load and to ensure the virus penetrates into the inner space of the tissue

The penetration rate was determined in the development of the manufacturing process.

Extraction Procedure

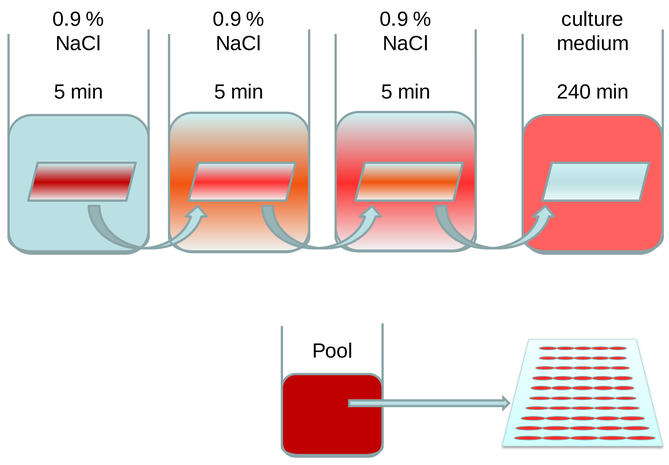
Before and after treatment the tissue was immersed in different solutions in order to:

- Release virus into the supernatant
- Reduce cytotoxicity of chemical left in the tissue after tissue collection

This was achieved with a stepwise approach :

- 3 x 5-6 min in 2 ml 0.9% NaCl
- 1 x 2 h in 4 ml cell culture medium
- pooling of wash solutions

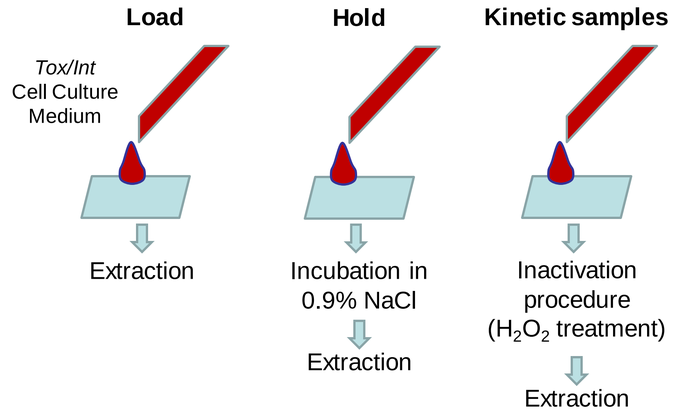
The duration of the extraction process did consider the penetration rate. The pool of all solutions (extract solution) was analyzed for virus.



Pre-test

Toxicity- and Interference assay

The extraction process was applied to untreated and H_2O_2 treated representative tissue pieces (unspiked!). The extract solutions were analyzed for cytotoxicty and interference.



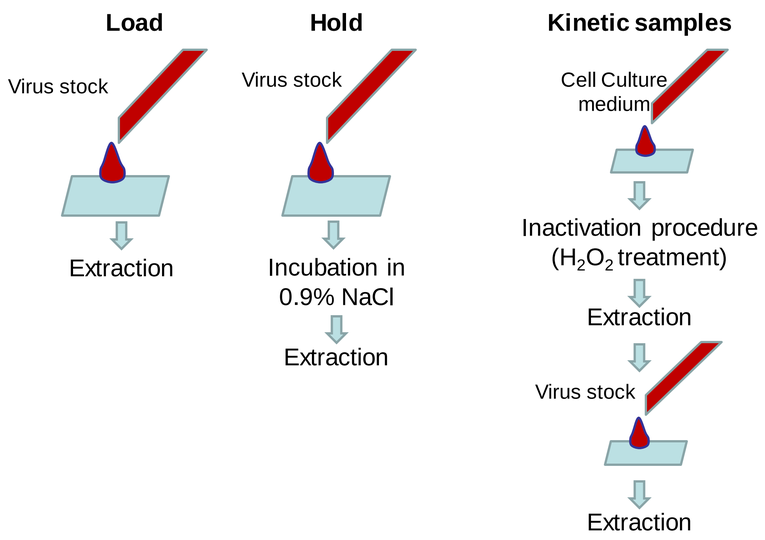
Tab. 1 minimal non-interfering dilutions (MD) and the log10 reduction factor

Representative material to	MD	MuLV	PRV	Reo-3	PPV
Load	**1:60**	0.30	- 0.06	0.00	- 0.42
Hold	**1:60**	- 0.12	- 0.06	- 0.12	- 0.12
Process sample	**1:486**	- 0.06	0.00	- 0.24	- 0.12

A non-interfering dilution was determined for each material. A higher dilution was necessary for the process samples containing H_2O_2.

Recovery assay

Untreated (Load and Hold) and H_2O_2 pre-treated tissue pieces were spiked with a pre-defined virus stock solution and incubated accordingly. The extract solutions derived from the subsequent extraction process were analyzed for the virus titer.



Tab. 2 Recovery assay: log_{10} total virus load

	MuLV	PRV	Reo-3	PPV
Theoretical virus load	5.97 ± 0.25	6.92 ± 0.26	7.21 ± 0.27	7.98 ± 0.25
Load	5.08 ± 0.26	6.59 ± 0.32	7.11 ± 0.32	8.24 ± 0.20
Hold	5.92 ± 0.30	6.39 ± 0.24	7.07 ± 0.24	7.71 ± 0.24
Process sample	5.58 ± 0.30	6.47 ± 0.25	7.84 ± 0.24	7.89 ± 0.24

Each virus was recovered from the tissue.

Inactivation process

Load

Spiking of tissue piece and immediate extraction.

Hold

Spiking of tissue piece, incubation in 0.9% NaCl until end of inactivation process and subsequent extraction.

Inactivation

Spiking of tissue piece, incubation in H_2O_2 and subsequent extraction. For each time point of the kinetic one piece of tissue was used.

Results

Tab. 3 H_2O_2 treatment: log_{10} total virus load

	MuLV		PRV		Reo-3		PPV	
run	1	2	1	2	1	2	1	2
Load	5.08	5.63	6.59	6.39	7.11	6.93	8.18	8.18
24 h	**≤ 3.06**	**≤ 3.13**	**2.68**	**≤ 3.18**	**≤ 3.30**	**≤ 3.40**	**≤ 3.69**	**≤ 3.69**
Hold	5.92	5.50	6.39	6.45	6.11	6.83	7.86	7.86
LRF (24 h)	**≥ 3.00**	**≥ 3.38**	**4.54**	**≥ 4.08**	**≥ 3.81**	**≥ 3.51**	**≥ 5.31**	**≥ 5.33**

The virus was inactivated down to the limit of detection for all processes besides for run 1 of PRV.

Conclusion

Viral clearance studies for medical devices include often solid phase material as test item. In such a case the right conditions for spiking into the solid material and the recovery of virus from the solid material is crucial.

The high cytotoxicity of process samples derived from harsh conditions like H_2O_2 treatment and the sometimes reduced virus recovery limit the dynamic range of LRF, which can be demonstrated even though the process step itself might have high inactivation or removal capacity.

The specific experimental conditions, sometimes in combination with a limited number of potential virus reduction step, will make it challenging to demonstrate the overall reduction currently requested in the FDA draft guidance on medical devices (2).

Literature

(1) Medical devices utilizing animal tissues and their derivatives. Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents, ISO 22442-3, December 2008

(2) Medical Devices Containing Materials Derived from Animal Sources (Except for In Vitro Diagnostic Devices) - Draft Guidance for Industry and Food and Drug Administration Staff, FDA, January 23, 2014.

Contact

Charles River
Biopharmaceutical Services GmbH
Oliver Schaefer, PhD.
tel.: +49 (0) 221-346180-217
mail: oliver.schaefer@crl.com

PDA Virus & TSE safety forum 2015
Portugal – Lisboa (Cascais)
Q22/2015