Inactivation of swine vesicular disease virus in porcine plasma by spray-drying

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Introduction

Published information on viral inactivation due to the manufacturing process of spray-dried plasma protein (SDP) is limited. Rapid changes in temperature and pressure during spray-drying cause instantaneous evaporation of water that is detrimental of survival of viruses. Swine vesicular disease virus (SVD) is a non-lipid enveloped virus that belongs to the *Pircornaviridae* family and is recognized to be highly resistant to physical and chemical processing conditions. Swine vesicular disease has stability characteristics similar to foot and mouth disease virus (FMD). Therefore, SVD was selected as a model to evaluate the effects of spray-drying conditions on viral inactivation.

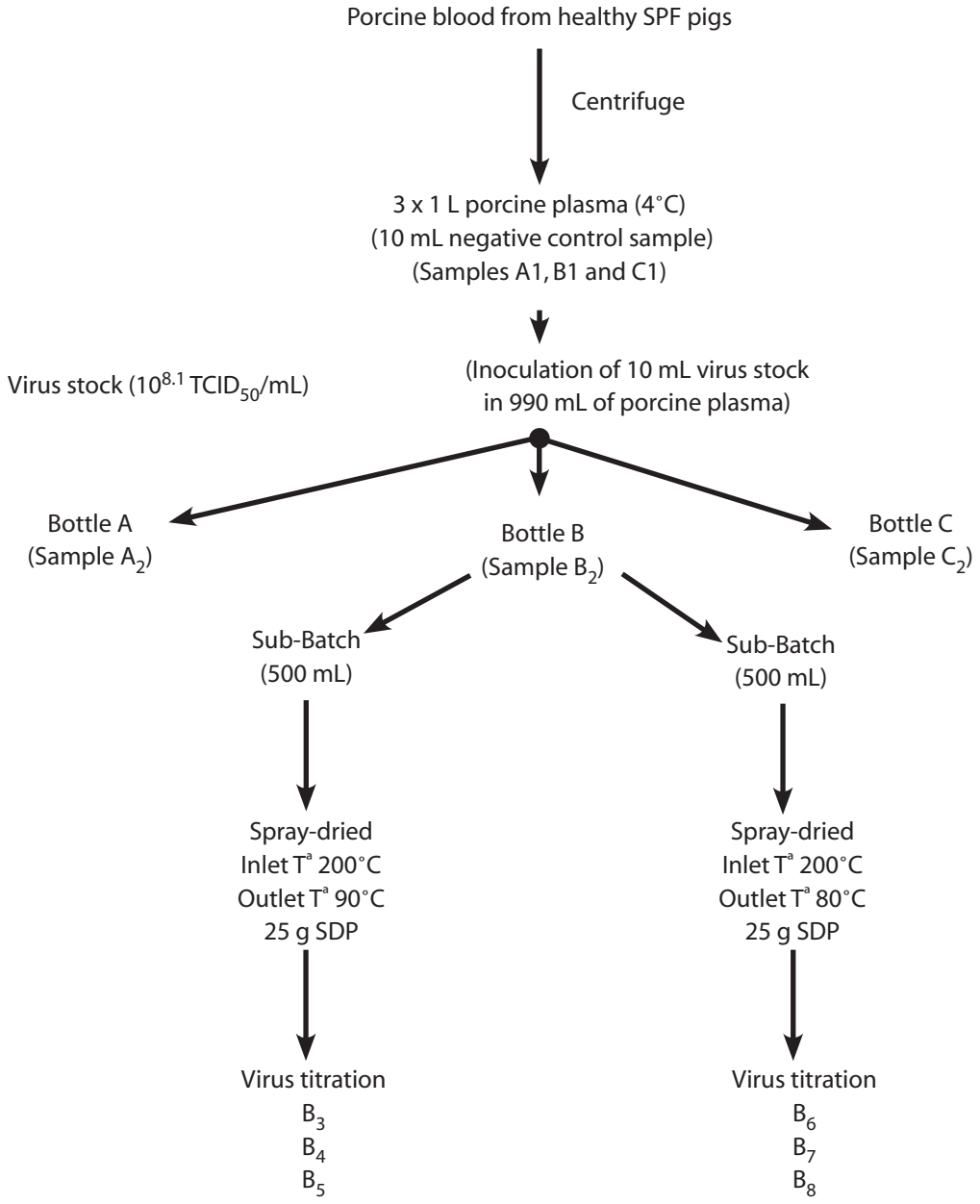
Materials and methods

Swine vesicular disease virus (strain UK72) was propagated in swine kidney cell line (IBRS-2 cell line). A SVD virus stock was produced on IBRS-2 cells to obtain a virus titer of 10^8 $TCID_{50}$/mL. Porcine plasma was collected from six SPF pigs in an EU inspected slaughter facility. Animals were previously inspected and approved for human consumption. Blood was collected into a container containing sodium phosphate as anticoagulant, and transported refrigerated to the APC Europe S.A. laboratory (Granollers, Spain) where the plasma was separated by centrifugation. Three 1-L bottles of liquid porcine plasma, derived from the same batch of plasma were prepared then transported to an isolation room in the BSL3 animal facility of CRESA (Spain). Previously, a 10-mL sample of each 1-L bottle was stored (-80°C) for use as a negative control to screen for SVD virus neutralization (VN) antibodies and viral contamination. Each bottle of plasma (990 mL) was inoculated with 10 mL of stock virus to obtain approximately 10^5 $TCID_{50}$ SVD/mL. A 10 mL sample was stored at -80°C for further virus titration as control of plasma infection. Each one of the 1-L SVD inoculated plasma bottles was divided into sub-samples of 500 mL and dried using a pilot plant spray-drier (Anhydro Compact Spray Dryer, Anhydro A/S, Copenhagen, DK). The suspension flow to the nozzle was 10 Lh^{-1}. Airflow through the feeding nozzle was 15 m^3h^{-1}. Air temperature was maintained at 20°C. The estimated dwell time was 0.41 s. One sub-sample was dried with an inlet air temperature of 200 ± 5°C and an outlet air temperature of 90 ± 1°C. The other sub-sample was dried at the same inlet temperature but the outlet air temperature was set at 80 ± 1°C (Figure 1). Samples of inoculated liquid plasma and spray-dried plasma were frozen with dry ice and stored at -80° prior to analysis of infectivity in IBRS-2 cell cultures, using a micro-titer assay procedure.[1] Dried plasma samples were reconstituted by adding 0.5 g dried sample to 5.55 mL of distilled water. To determine that no minute quantities of virus survived, 4 mL of each of the spray-dried samples were inoculated on the 75 cm^2 flask of IBRS-2 cells. After three to four days, cell cultures were harvested and passed to new IBRS-2 cell cultures. Four serial passages were made. The theoretical limit of detection for this method was 0.25 viral particles per mL.

Results and discussion

The negative control sample (Figure 1), did not contain any detectable citopatic effect producing virus or antibody titer for SVD, pseudorabies virus (PRV) and porcine parvovirus (PPV). The negative control sample was also subjected to four serial passages on IBRS-2 cell line and results were negative for SVD. The SVD virus stock solution used to infect the three bottles of plasma yielded a virus titer of $10^{8.1}$ $TCID_{50}$/mL. The inoculated 1 L bottles of plasma (A, B, C) averaged $10^{5.64\pm0.2}$ $TCID_{50}$ SVD titer/mL, and individual samples ranged from $10^{5.42}$ to $10^{6.00}$ $TCID_{50}$ SVD titer/mL. All dried samples, regardless of the spray-drying conditions used, yielded negative results by micro-titer assays. After this step, 4 mL of 9% plasma suspension from all treatments were inoculated to 75 cm^2 culture bottles of IBRS-2 cell line. After a series of four passages, in IBRS-2 cellular line, all samples were negative. These results were considered negative because the isolation process was done in triplicate on each spray-drying condition and

Figure 1



in triplicate for each spray-dried sample (9 times on each treatment, Figure 1). A minimum reduction factor of $10^{5.62}$ $TCID_{50}$/mL of sample (0.363 g) was obtained from these results. Therefore, the spray-drying treatment inactivated about $10^{6.68}$ $TCID_{50}$/g of dehydrated plasma. The detection limits with a confidence of 95% obtained was below 100 $TCID_{50}$/L or 0.1 $TCID_{50}$/mL.

Both thermal inactivation and inactivation by dehydration are mechanisms that contribute to microbial mortality during the spray-drying process.[2, 3, 4] Both mechanisms occur simultaneously and have differential effects on microbial survival depending on the inherent resistance of the microorganism to heat or dehydration. Some microorganisms adapt to high temperature. However, the short drying time with the almost immediate increase in temperature does not allow enough time for the microorganism to adapt to this high temperature.[5] Cell damage or mortality caused by thermal

Table 1: Swine vesicular disease virus (SVD) infectivity of inoculated porcine plasma samples before and after spray-drying.

Lot # sample identification	Inoculated liquid plasma samples	Drying conditions	Spray-dried samples[1,2]
	TCID50/mL[3]		$TCID_{50}$/mL
Bottle A	$10^{5.42} – 10^{5.57}$	Inlet 200°C, Outlet 90°C	Not detected[4]
		Inlet 200°C, Outlet 80°C	Not detected
Bottle B	$10^{5.49} – 10^{5.67}$	Inlet 200°C, Outlet 90°C	Not detected
		Inlet 200°C, Outlet 80°C	Not detected
Bottle C	$10^{5.70} – 10^{6.00}$	Inlet 200°C, Outlet 90°C	Not detected
		Inlet 200°C, Outlet 80°C	Not detected
Average	$10^{5.64}$		Absence

1 Dried plasma samples were reconstituted by adding 0.5 g of dried sample to 5.55 mL of distilled water.

2 Reconstituted spray-dried samples (4 mL, 0.363 g) were passed four consecutive times in IBRS-2 cells to amplify any viable SVD virus which may have been undetected in the first pass.

3 Tissue culture infection dose as determined by micro-titer assay procedure (Burleson et al., 1997).

4 The theoretical limit of detection was 0.1 TCID50/mL.

inactivation is due to an effect on DNA, RNA (including ribosomal RNA), proteins (enzymes) and the cell membrane.[3]

Viral inactivation is essential to establish the safety of products of animal origin. Currently, the best validation procedure for viral inactivation is to deliberately contaminate a product with a known virus before the inactivation process then attempt to isolate live virus after processing. Due to regulatory restrictions on use of FMD in such studies as ours, it was necessary to select a less contagious virus such as SVD to evaluate the effects of spray-drying conditions on viral inactivation. Swine vesicular disease was selected as a model virus for our study because it belongs to the same viral family as FMD and shares its high physicochemical resistance characteristics.

The results suggest that the spray-drying conditions achieved greater than 6 log reduction of SVD in the inoculated porcine plasma samples. These results agree with other published results[6] using a similar model for Pseudorabies virus (PRV) and porcine respiratory and reproductive syndrome virus (PRRSV). No live PRV or PRRSV were detected in processed samples which indicated at least a 5 log reduction for PRV and a 4 log reduction for PRRSV in inoculated plasma spray-dried under similar conditions.

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