

Over the past 20 years, APC, Inc. has developed and refined techniques associated with the manufacturing process to ensure that spray-dried plasma proteins are biologically safe and functional for use in nutritional supplements for swine, companion animals, ruminants, poultry, and aquaculture. Our dedication to research has led to many new discoveries and scientific publications as to how plasma proteins function to benefit animals in various applications.

Recently, spray-dried porcine plasma has been implicated as a vector responsible for the introduction of PED virus into Canada. Further scientific investigation indicates that environmental contamination is a more likely explanation.

Executive Summary

APC has completed a series of experiments to determine the biosafety of spray-dried porcine plasma relative to porcine epidemic diarrhea virus (PEDv). The results of some of these experiments reported herein indicate the following:

- PED virus does not survive spray-drying.
- Experimentally inoculated spray-dried plasma loses infectivity by 7 days when stored at room temperature.
- Results from an APC sponsored pig bioassay demonstrate that two retained samples from the lot of spray-dried porcine plasma investigated by CFIA and two other samples of PCR-positive commercial spray-dried porcine plasma did not contain infective PED virus.
- Independent of APC research, the FDA acquired samples of the specific lot of spray-dried porcine plasma investigated by the CFIA. Three of these samples, corresponding to the CFIA samples, were prepared and inoculated into 17-19 day old piglets (5 pigs/lot). **All three samples were negative by bioassay.** The FDA results were consistent with the results of the bioassays conducted by APC.
- Weaned pigs fed a diet with 5% PCR-positive commercial spray-dried porcine plasma for 14 days after weaning remained PEDv negative for the 21 day feeding period as determined by absence of serum antibodies against PEDv and by negative PCR analysis of fecal swabs and intestinal contents for PEDv.

Post-processing storage and certification

APC is initiating a redundant safety step for the production of spray-dried porcine plasma. APC will certify that spray-dried porcine plasma has been stored at a minimum of 70°F (21°C) for 14 days prior to release for sale. In addition, APC will provide validation data to support this post-processing storage step.

Additional information will be reported from other studies in progress as final results become available.

EXPERIMENT 1

Objective: Test spray-drying capacity to inactivate PEDv.

Results:

Sample ID	Spray-Dried TCID ₅₀ /g
Inlet 200°C / Outlet 80°C	
Post-processing 30 sec @ 70°C inner temperature	Not detected
Post-processing 60 sec @ 80°C inner temperature	Not detected

Estimated reduction of PEDv infectivity in spray-dried plasma was calculated to be $> 10^{4.5}$ TCID₅₀/g

Conclusion: In this experiment, PED virus did not survive the simulated commercial spray-drying conditions.

Experiment 1 was conducted at the Centre de Recerca en Sanitat Animal (CRESA), Barcelona, Spain.

EXPERIMENT 2.1

Objective: Test storage temperature conditions to inactivate PEDV.

Results:

Survival of PEDv in Spray Dried Bovine Plasma held at Room Temperature		
Day of Storage	0	7
PEDv Detection	Positive ¹	Negative

¹PED virus concentration on spray-dried plasma was 1×10^3 TCID₅₀/g.

Conclusion: PED virus does not survive on spray-dried plasma for more than 7 days when stored at room temperature.

EXPERIMENT 2.2

Objective: Test time and storage temperature conditions to inactivate PEDv

Preliminary Results:

Survival of PEDv on Spray Dried Bovine Plasma held at Various Time by Temperature Conditions ¹			
Day of Storage	Storage Temperature		
	4°C	12°C	21°C
7	Positive	Positive	Negative
14	Positive	Negative	Negative
21	Negative	Negative	Negative

¹PED virus concentration on spray-dried plasma was $1 \times 10^{2.8}$ TCID₅₀/g.

Conclusion:

- At 4°C storage temperature, PED virus survived on spray-dried plasma for 14 days but did not survive when stored for 21 days.
- At 12°C storage temperature, PED virus survived on spray-dried plasma for 7 days but did not survive when stored for 14 or 21 days.
- At 21°C room temperature storage, PED virus did not survive on spray-dried plasma for 7, 14 or 21 days.
- Survival of PED virus inoculated on spray-dried plasma is storage time by temperature dependent with longer survival time in colder temperature.

Experiments 2.1 and 2.2 were conducted at the Centre de Recerca en Sanitat Animal (CRESA), Barcelona, Spain.

EXPERIMENT 3

Objective: Bioassay with commercial spray-dried porcine plasma.

Results:

Sample ID	Initial PEDv PCR (CT)	Clinical signs 72 h post inoculation ¹			Jejunum PEDv RT-PCR (CT)			IHC
		Pig 1	Pig 2	Pig 3	Pig 1	Pig 2	Pig 3	
Sample 1 ²	30.12	0	0	0	Neg.	Neg.	Neg.	Neg.
Sample 2 ²	31.28	0	0	0	Neg.	Neg.	Neg.	Neg.
Sample 3 ³	29.86	0	0	1	Neg.	Neg.	Neg.	Neg.
Sample 4 ³	30.71	0	0	0	Neg.	Neg.	Neg.	Neg.
Positive Control	18.61	3	3	3	16.53	15.30	15.30	Pos.
Negative Control	-	0	0	0	Neg.	Neg.	Neg.	Neg.

¹Score 0 = no clinical signs well-formed solid feces; score 1 = soft poorly formed feces; score 2 = presence of watery liquid feces; score = watery diarrhea and dehydration.

²Samples 1 and 2 were from the retained sample of the plasma in the CFIA investigation.

³Samples 3 and 4 were randomly selected commercial product.

Experiment 3 was conducted at the Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN.

FDA RESEARCH

Results of FDA Bioassays

	Sample PCR	Bioassay ¹	
		PCR	Serology
Sample 1	Positive	Negative	Negative
Sample 2	Positive	Negative	Negative
Sample 3	Positive	Negative	Negative

¹Three samples, corresponding to the CFIA samples, were prepared and inoculated into 17-19 day old piglets (5 pigs/lot).

FDA Report on Bioassay Analysis:

“Please share, In summary: NVSL received ten (10) porcine plasma samples collected by the FDA from the US plasma producer. These samples were tested by PCR for the presence of PEDV RNA and nine of the samples were positive, while one is still pending. Three of these samples, corresponding to the CFIA samples, were prepared and inoculated into 17-19 day old piglets (5 pigs/lot). **All three samples were negative by bioassay.**”

FDA Research was conducted at the National Veterinary Services Laboratory in Ames, IA.

Conclusions: Commercial spray-dried porcine plasma, PCR positive for PEDv genome, did not transmit PEDv to naïve pigs. Also, the two retained samples from the lot of plasma investigated by CFIA in the Ontario cases did not transmit PEDv to naïve pigs.

FDA Research also confirmed that three samples corresponding to the CFIA samples were also negative by bioassay.

EXPERIMENT 4

Objective: Feeding spray-dried porcine plasma PCR positive for PEDv genome in a meal diet to conventional weaned pigs

Forty-eight pigs weaned at an average of 21 days of age were randomly distributed into two different treatment groups with 6 pens per dietary treatment (Trt) and 4 pigs per pen.

Results:

Analysis	Pigs in Control Group	Pigs in Plasma Group
PCR in fecal samples at day 0	NEGATIVE	NEGATIVE
PCR in fecal samples at day 3	NEGATIVE	NEGATIVE
PCR in fecal samples at day 7	NEGATIVE	NEGATIVE
PCR in fecal samples at day 14	NEGATIVE	NEGATIVE
PCR in fecal samples at day 21	NEGATIVE	NEGATIVE
PCR in intestinal tissue at day 21	NEGATIVE	NEGATIVE
Serum antibodies against PEDv at day 21	NEGATIVE	NEGATIVE

- Fecal swabs of all pigs in both groups were PCR negative for PEDv at day 0, 3, 7, 14, and 21.
- Intestinal content of all pigs at day 21 were PCR negative for PEDv.
- All pigs in both groups at day 21 did not have antibodies against PEDv demonstrating that there was no seroconversion against PEDv and that these pigs had not been exposed to live PED virus.

Conclusion: Feeding pigs a diet containing 5% commercial spray-dried porcine plasma that was PCR positive for PEDv did not demonstrate any evidence of PEDv infectivity through 21 days post-weaning.

Experiment 4 was conducted at a contract research facility under the management of trained technicians and veterinary supervision.

Overall conclusions of these studies indicate that environmental contamination is a more likely explanation for the CFIA test results, and also that PCR Positive results do not indicate a potential PEDv infection.

To learn more about our products call APC 800-513-8755, fax 515-289-7600,
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