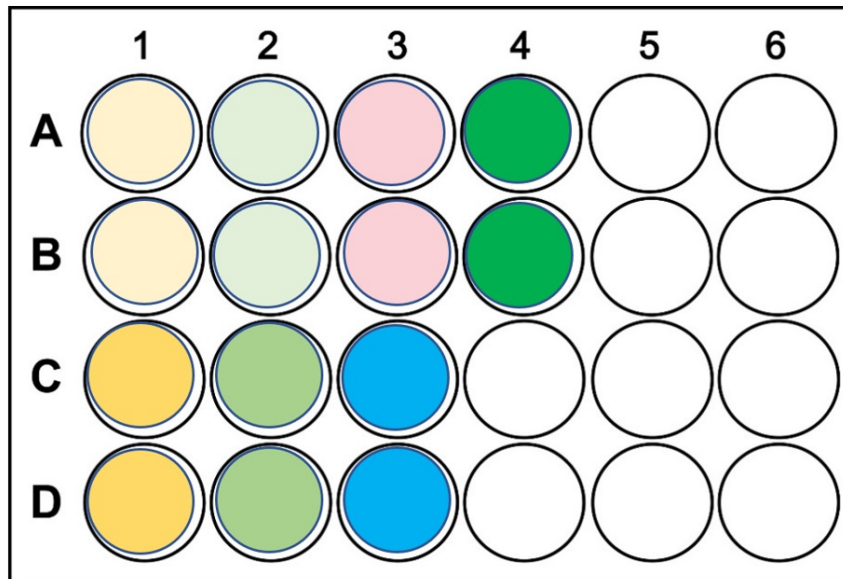


2023-11 XB In vitro study

2ml of 1×10^5 HAD₅₀/ml ASFV were mixed with 1 oz, 2oz and 4oz/gallon concentrations of XB High Foam (1ml added at a time, each with 10 min contact time, repeated three times) with final volume of ~ 5ml. Mixtures were diluted 1:20 in media (previously determined by toxicity assay) and cultured in PB-derived macrophages for 5 days. Day 0 and Day 5 samples were collected for RT-PCR ASF detection.

Plate set-up



Legend:

- Yellow circle: XB HF 1.0
- Orange circle: XB HF 2.0
- Light Green circle: XB HF 4.0
- Medium Green circle: PBS, no ASFV
- Pink circle: PBS + ASFV
- Blue circle: Media only
- Dark Green circle: ASF only (undiluted)

2023-11-13 RT-PCR plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S Target... 4E6 Ct: 22.04	S Target... 4E6 Ct: 21.81	D0 1HF.1 U Target... 3.62E3 Ct: 33.3	D0 1HF.1 U Target... 3.06E3 Ct: 33.58	D0 PBS+A... U Target... 4.81E3 Ct: 32.84	D0 PBS+A... U Target... 5.3E3 Ct: 32.68	D5 2HF.1 U Target... 4.9E3 Ct: 32.81	D5 2HF.1 U Target... 5.22E3 Ct: 32.7	D5 media.1 U Target... 5.39E3 Ct: 32.65	D5 media.1 U Target... 4.49E3 Ct: 32.95		
B	S Target... 4E5 Ct: 25.4	S Target... 4E5 Ct: 25.25	D0 1HF.2 U Target... 3.86E3 Ct: 33.2	D0 1HF.2 U Target... 3.8E3 Ct: 33.22	D0 PBS+A... U Target... 6.3E3 Ct: 32.39	D0 PBS+A... U Target... 5.78E3 Ct: 32.53	D5 2HF.2 U Target... 4.03E3 Ct: 33.13	D5 2HF.2 U Target... 3.75E3 Ct: 33.25	D5 media.2 U Target... 1.95E3 Ct: 34.32	D5 media.2 U Target... 2.07E3 Ct: 34.23		
C	S Target... 4E4 Ct: 29.26	S Target... 4E4 Ct: 29.19	D0 2HF.1 U Target... 4E3 Ct: 33.14	D0 2HF.1 U Target... 4.29E3 Ct: 33.02	2 D0 media.1 U Target 1 Ct: Undete	2 D0 media.1 U Target 1 Ct: Undete	D5 4HF.1 U Target... 8.03E3 Ct: 31.99	D5 4HF.1 U Target... 6.7E3 Ct: 32.29	D5 ASF onl... U Target... 2.59E7 Ct: 18.72	D5 ASF onl... U Target... 3.2E7 Ct: 18.37		
D	S Target... 4E3 Ct: 33.17	S Target... 4E3 Ct: 32.96	D0 2HF.2 U Target... 4.16E3 Ct: 33.08	D0 2HF.2 U Target... 3.93E3 Ct: 33.17	2 D0 media.2 U Target 1 Ct: Undete	2 D0 media.2 U Target 1 Ct: Undete	D5 4HF.2 U Target... 7.16E3 Ct: 32.18	D5 4HF.2 U Target... 6.43E3 Ct: 32.36	D5 ASF onl... U Target... 2.99E7 Ct: 18.48	D5 ASF onl... U Target... 2.92E7 Ct: 18.52		
E	1 S Target... 400 Ct: 37.36	1 S Target... 400 Ct: 38.13	D0 4HF.1 U Target... 2.87E3 Ct: 33.68	D0 4HF.1 U Target... 4.36E3 Ct: 33	D0 ASF onl... U Target... 1.03E5 Ct: 27.8	D0 ASF onl... U Target... 1.2E5 Ct: 27.54	D5 PBS no... U Target... 63.37 Ct: 39.95	1 D5 PBS no... U Target 1 Ct: Undete				
F	S Target... 40 Ct: 40.27	S Target... 40 Ct: 40.13	D0 4HF.2 U Target... 4.94E3 Ct: 32.79	D0 4HF.2 U Target... 3.64E3 Ct: 33.3	D0 ASF onl... U Target... 9.33E4 Ct: 27.96	D0 ASF onl... U Target... 1.1E5 Ct: 27.69	D5 PBS no... U Target... 67.09 Ct: 39.86	2 D5 PBS no... U Target 1 Ct: Undete				
G	N Target 1 Ct: Undete	N Target 1 Ct: Undete	2 D0 PBS no... U Target 1 Ct: Undete	2 D0 PBS no... U Target 1 Ct: Undete	D5 1HF.1 U Target... 1.73E3 Ct: 34.52	D5 1HF.1 U Target... 2.39E3 Ct: 33.98	D5 PBS+A... U Target... 3.11E7 Ct: 18.42	D5 PBS+A... U Target... 3.15E7 Ct: 18.4				
H	N Target 1 Ct: Undete	N Target 1 Ct: Undete	2 D0 PBS no... U Target 1 Ct: Undete	2 D0 PBS no... U Target 1 Ct: Undete	D5 1HF.2 U Target... 2.38E3 Ct: 33.99	D5 1HF.2 U Target... 3.23E3 Ct: 33.49	D5 PBS+A... U Target... 2.72E7 Ct: 18.64	D5 PBS+A... U Target... 2.8E7 Ct: 18.59				

RT-PCR standard results

2023-11-13 XB ASF RT-PCR Results				HAD50/ml
Sample	Ct	Ct Mean	Ct SD	Quantity
Standard	22.04	21.93	0.16	4,000,000
Standard	21.81	21.93	0.16	4,000,000
Standard	25.40	25.32	0.11	400,000
Standard	25.25	25.32	0.11	400,000
Standard	29.26	29.22	0.05	40,000
Standard	29.19	29.22	0.05	40,000
Standard	33.17	33.06	0.15	4,000
Standard	32.96	33.06	0.15	4,000
Standard	37.36	37.75	0.54	400
Standard	38.13	37.75	0.54	400
Standard	40.27	40.20	0.10	40
Standard	40.13	40.20	0.10	40
NTC	Undetermined			0
NTC	Undetermined			0

2023-11-13 ASF RT-PCR Day 0 vs Day 5 results

Day 0						Day 5					
Sample	Ct	Ct Mean	Ct SD	Quantity	Qty Mean	Sample	Ct	Ct Mean	Ct SD	Quantity	Qty Mean
D0 1HF.1	33.30	33.44	0.20	3,622.98	3,341.59	D5 1HF.1	34.52	34.25	0.38	1,729.37	2,061.40
	33.58						33.98				
D0 1HF.2	33.20	33.21	0.02	3,858.45	3,830.64	D5 1HF.2	33.99	33.74	0.36	2,380.33	2,806.51
	33.22						33.49				
D0 2HF.1	33.14	33.08	0.08	3,998.14	4,143.47	D5 2HF.1	32.81	32.75	0.07	4,900.97	5,059.22
	33.02						32.70				
D0 2HF.2	33.08	33.12	0.06	4,157.62	4,044.77	D5 2HF.2	33.13	33.19	0.09	4,034.77	3,890.06
	33.17						33.25				
D0 4HF.1	33.68	33.34	0.49	2,871.57	3,617.16	D5 4HF.1	31.99	32.14	0.21	8,029.34	7,364.90
	33.00						32.29				
D0 4HF.2	32.79	33.04	0.36	4,938.97	4,288.28	D5 4HF.2	32.18	32.27	0.12	7,161.51	6,797.46
	33.30						32.36				
D0 PBS no ASF.1	Undetermined			0.00		D5 PBS no ASF.1	39.95	39.95		63.37	63.37
	Undetermined			0.00			Undetermined			0.00	
D0 PBS no ASF.2	Undetermined			0.00		D5 PBS no ASF.2	39.86	39.86		67.09	67.09
	Undetermined			0.00			Undetermined			0.00	
D0 PBS+ASF.1	32.84	32.76	0.11	4,812.69	5,054.03	D5 PBS+ASF.1	18.42	18.41	0.01	31,109,678.00	31,282,852.00
	32.68						18.40				
D0 PBS+ASF.2	32.39	32.46	0.10	6,297.20	6,037.50	D5 PBS+ASF.2	18.64	18.61	0.03	27,179,542.00	27,586,960.00
	32.53						18.59				
D0 media.1	Undetermined			0.00		D5 media.1	32.65	32.80	0.21	5,390.72	4,941.00
	Undetermined			0.00			32.95				
D0 media.2	Undetermined			0.00		D5 media.2	34.32	34.27	0.06	1,953.54	2,009.40
	Undetermined			0.00			34.23				
D0 ASF only.1	27.80	27.67	0.18	102,840.67	111,620.33	D5 ASF only.1	18.72	18.54	0.25	25,906,824.00	28,976,852.00
	27.54						18.37				
D0 ASF only.2	27.96	27.83	0.19	93,276.80	101,781.00	D5 ASF only.2	18.48	18.50	0.03	29,904,342.00	29,548,848.00
	27.69						18.52				

Legend:
 HF - XB High Foam at 1oz (1HF), 2oz (2HF) and 4oz (4HF) per gallon
 PBS - XB negative control, no XB without ASF (no ASF) or ASF added (+ASF)
 ASF only - undiluted virus stock/no XB or PBS added
 media - negative control

Results:
 The presence of the 1:20 diluted XB/ASFv mix appear to have a inhibiting effect on ASFv growth after 5 days in culture. 1oz/gal XBHF appear to be most inhibiting
 After 5 days in culture, ASFv is detected in all treatment groups but appear to be drastically reduced compared to the absence of XB High Foam (PBS+ASF and ASF only).