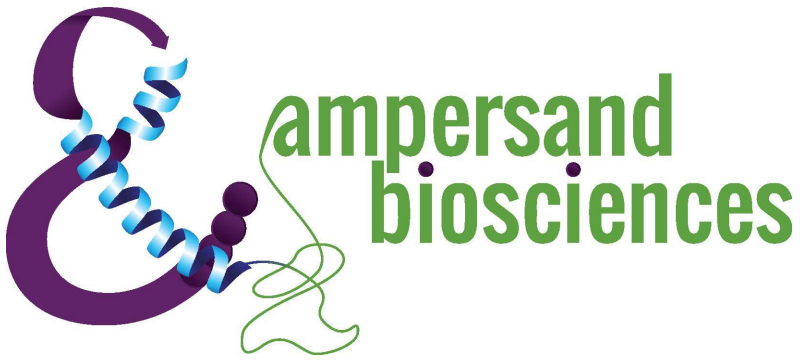


Porcine Cytokine Panel 1 (9-Plex)

Kit # P117-K

Validation Report



Prepared by: Marc Damour
Marc Damour (Jun 9, 2025 15:24 EDT)

Date 06/09/25

Reviewed by: Laurie Stephen

Date 06/09/25

1. Assay Description:

A multiplex assay was developed and validated for the measurement of IFN α , IFN γ , IL-1 β , IL-6, IL-8, IP-10, MDC, MIP-1 β , and TNF α . The kit is microsphere-based and consist of using antigen-specific antibodies covalently coupled to magnetic Luminex[®] beads and biotinylated detection antibodies in a capture-sandwich format. Incubations take place at 4°C and room temperature in a 96-well plate. 30 μ L of standard, controls or sample (diluted 1:3) are added to the appropriate wells, followed by 10 μ L of blocker and 10 μ L of multiplexed capture-antibody microspheres. The plate is incubated overnight (16-18hrs) at 4°C on a plate shaker. After washing 3 times, 40 μ L of detection antibodies are added to each well, thoroughly mixed, and incubated 1 hour at ambient temperature on a plate shaker. The Streptavidin-Phycoerythrin conjugate (SA-PE) working solution is then added to the plate and incubated for 30 minutes. The plate is then washed 3 times and the beads are resuspended in 100 μ L of wash buffer. After shaking on a plate shaker for 10 minutes, the plate is then analyzed on the Luminex[®] 200 Analyzer using the standard PMT setting.

2. Control and Sample Description:

Control	Description
Control 1	Normal Porcine Serum (22%) spiked with Recombinant IFN α , IFN γ , IL-1 β , IL-6, IL-8, MDC, MIP-1 β , and TNF α .
Control 2	Normal Porcine Serum (3%) spiked with Recombinant IFN α , IFN γ , IL-1 β , IL-6, IL-8, MDC, MIP-1 β , and TNF α .

Sample	Description
Serum 1	Normal Porcine Serum spiked with recombinant antigens IFN γ , IL-1 β , IL-6, MDC, MIP-1 β .
Serum 2	Normal Porcine Serum spiked with recombinant antigens IFN γ , IL-1 β , IL-6, MDC, MIP-1 β .
Plasma 1	Normal Porcine Plasma spiked with recombinant antigens IFN γ , IL-1 β , IL-6, MDC, MIP-1 β .
Plasma 2	Normal Porcine Plasma spiked with recombinant antigens IFN γ , IL-1 β , IL-6, MDC, MIP-1 β .

3. LLOQ, LDD and Curves:

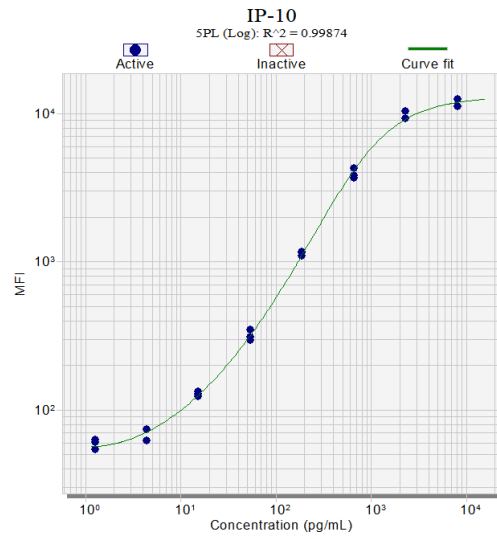
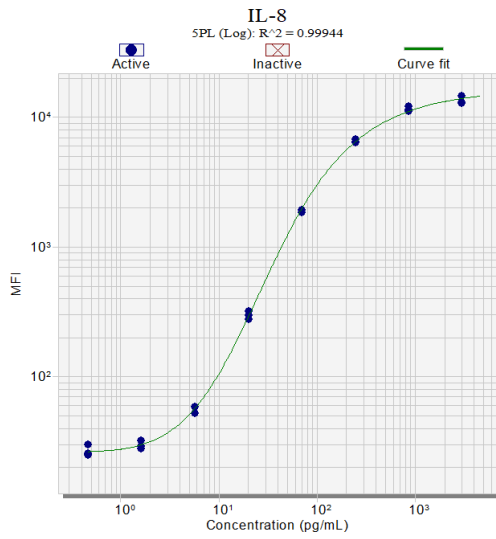
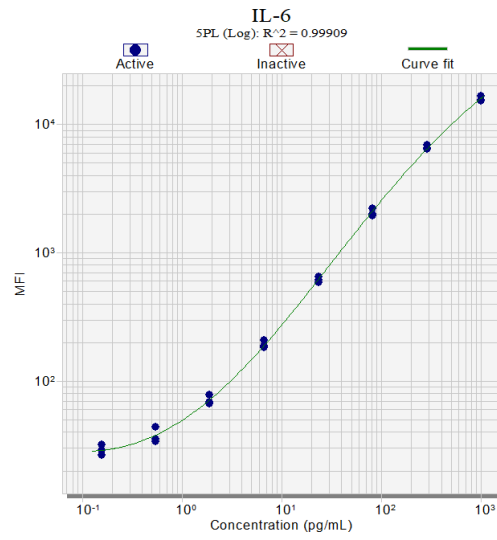
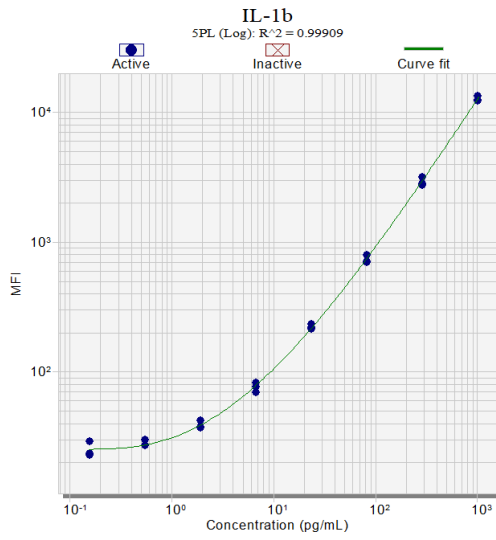
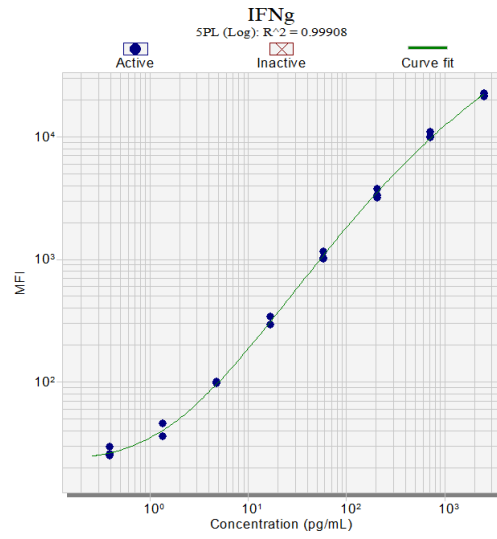
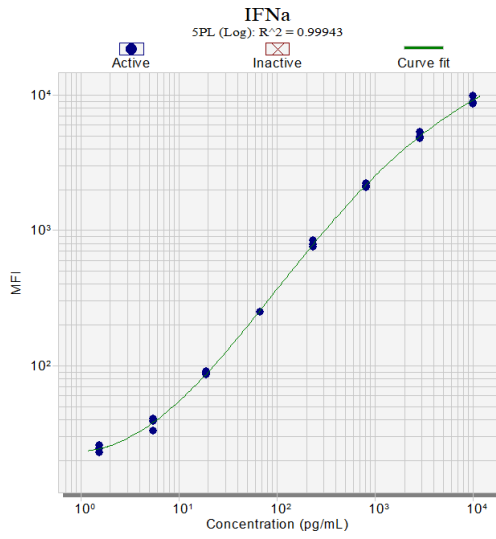
LDD: MFI (Median Fluorescent Intensity) for 10 replicates of the standard curve diluent was averaged and two (2) standard deviations added. This value was calculated to concentration off the standard curve.

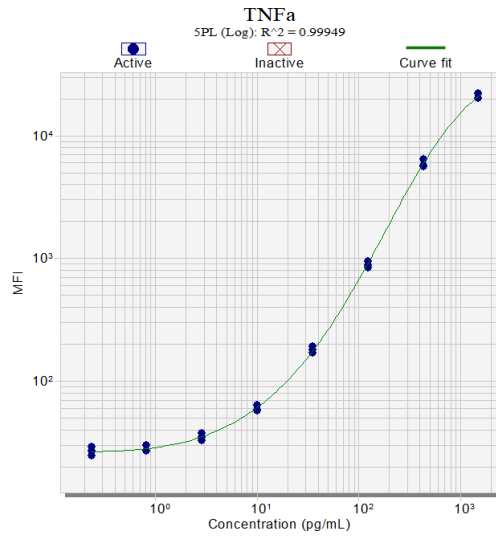
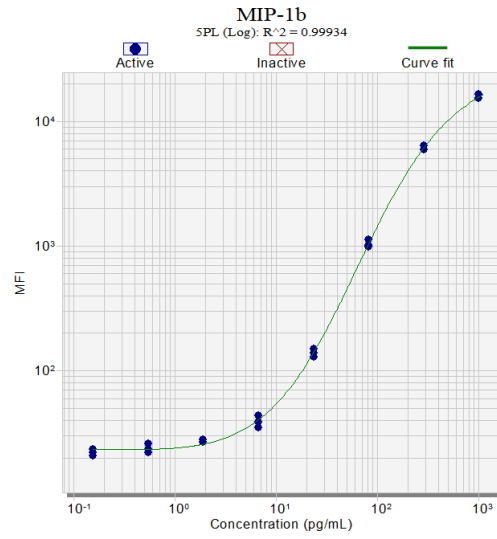
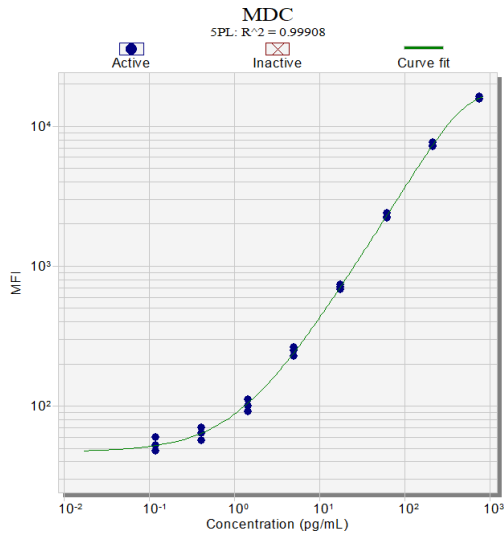
LLOQ: LLOQ was assessed by diluting a low serum sample for 8, 2-fold dilutions in triplicate. The LLOQ represents the value at which 30% CV was attained, with linearity with 70-130%. If that value calculates lower than the LOD, then the LLOQ value is equal to the LOD.

Curves: Curves were calculated using the best fit function in Plate Viewer Software. The S1 is the lowest level standard and the S8 is the highest.

		S1	S8	LDD	LLOQ
IFN α	pg/mL	1.6	10000	2.0	2.3
IFN γ	pg/mL	0.39	2500	0.39	0.48
IL-1 β	pg/mL	0.16	1000	1.0	1.0
IL-6	pg/mL	0.16	1000	0.32	0.46
IL-8	pg/mL	0.47	3000	1.8	1.8
IP-10	pg/mL	1.2	8000	3.1	3.1
MDC	pg/mL	0.12	750	0.42	0.51
MIP-1 β	pg/mL	0.16	1000	3.5	3.5
TNF α	pg/mL	0.23	1500	2.1	2.3

X dilution factor		S1	S8	LDD	LLOQ
IFN α	pg/mL	4.7	30000	5.9	7.0
IFN γ	pg/mL	1.2	7500	1.2	1.4
IL-1 β	pg/mL	0.47	3000	2.9	2.9
IL-6	pg/mL	0.47	3000	1.0	1.4
IL-8	pg/mL	1.4	9000	5.5	5.5
IP-10	pg/mL	3.7	24000	9.2	9.2
MDC	pg/mL	0.35	2250	1.3	1.5
MIP-1 β	pg/mL	0.47	3000	10	10
TNF α	pg/mL	0.70	4500	6.2	6.8





4. Precision:

Control samples were run in triplicate over 3 runs over 2 days with 2 analysts. Precision is the % CV of each run (intra, each run; inter, over 3 runs). Acceptance for precision is <20% CV. All assays meet acceptance for precision.

IFN α (pg/mL)		Run 1	Run 2	Run 3	Inter
Control 1	Mean	172	151	162	162
	% CV	2%	8%	9%	8%
Control 2	Mean	933	720	902	852
	% CV	9%	9%	8%	12%

IFN γ (pg/mL)		Run 1	Run 2	Run 3	Inter
Control 1	Mean	31	28	27	29
	% CV	0%	3%	11%	8%
Control 2	Mean	222	187	217	209
	% CV	2%	2%	1%	8%

IL-1 β (pg/mL)		Run 1	Run 2	Run 3	Inter
Control 1	Mean	39	37	40	39
	% CV	2%	4%	5%	5%
Control 2	Mean	374	386	373	378
	% CV	1%	2%	2%	2%

IL-6 (pg/mL)		Run 1	Run 2	Run 3	Inter
Control 1	Mean	44	36	39	40
	% CV	2%	6%	5%	10%
Control 2	Mean	165	127	158	150
	% CV	1%	2%	1%	12%

IL-8 (pg/mL)		Run 1	Run 2	Run 3	Inter
Control 1	Mean	27	24	25	25
	% CV	2%	4%	3%	6%
Control 2	Mean	125	108	122	118
	% CV	1%	2%	1%	7%

IP-10 (pg/mL)		Run 1	Run 2	Run 3	Inter
Control 1	Mean	128	130	109	123
	% CV	3%	5%	9%	10%
Control 2	Mean	1130	949	1073	1051
	% CV	2%	6%	2%	8%

MDC (pg/mL)		Run 1	Run 2	Run 3	Inter
Control 1	Mean	3.5	3.8	3.3	3.5
	% CV	9%	7%	14%	11%
Control 2	Mean	31	28	29	30
	% CV	4%	7%	3%	6%

MIP-1 β (pg/mL)		Run 1	Run 2	Run 3	Inter
Control 1	Mean	41	35	39	38
	% CV	2%	6%	4%	8%
Control 2	Mean	183	161	183	175
	% CV	2%	1%	1%	6%

TNF α (pg/mL)		Run 1	Run 2	Run 3	Inter
Control 1	Mean	66	55	61	61
	% CV	1%	6%	7%	9%
Control 2	Mean	269	224	267	253
	% CV	2%	1%	1%	9%

5. Linearity:

Linearity was assessed using 2 serum and 2 plasma samples spiked with standard and diluted 1:2 for 3 dilutions following the kit dilution. Percent Recovery was calculated using the calculated value (with kit dilution) as expected (observed x dilution / expected concentration X 100). The acceptance range for linearity is 70-130% recovery for all values above the LLOQ. All assays meet acceptance criteria.

IFN α pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	2460	2815	3110	3065
1:6	1420	1530	1570	1485
1:12	720	751	896	688
1:24	389	405	498	415
6	115%	109%	101%	97%
12	117%	107%	115%	90%
24	126%	115%	128%	108%

IFN γ				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	54	44	33	40
1:6	28	24	17	17
1:12	16	13	9.2	6.9
1:24	6.8	6.1	4.7	4.2
6	103%	107%	99%	88%
12	117%	116%	111%	70%
24	100%	111%	112%	84%

IL-1 β				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	2250	2120	372	306
1:6	1120	963	181	142
1:12	539	466	86	69
1:24	268	244	46	39
6	100%	91%	97%	93%
12	96%	88%	93%	90%
24	95%	92%	98%	102%

IL-6				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	76	97	10	705
1:6	38	47	4.1	333
1:12	22	24	2.4	197
1:24	12	13	<LLOQ	114
6	100%	97%	79%	94%
12	115%	101%	94%	112%
24	129%	110%	<LLOQ	129%

IL-8				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	2060	282	220	388
1:6	1105	122	125	210
1:12	476	70	63	106
1:24	236	45	32	59
6	107%	87%	114%	108%
12	92%	99%	115%	109%
24	91%	127%	117%	121%

IP-10				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	6995	4480	1050	1210
1:6	2895	2180	585	677
1:12	1370	1090	295	297
1:24	723	565	167	172
6	83%	97%	111%	112%
12	78%	97%	112%	98%
24	83%	101%	127%	113%

MDC				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	160	129	123	8.2
1:6	80	75	68	5.2
1:12	39	36	34	2.6
1:24	24	19	20	1.3
6	101%	116%	111%	127%
12	98%	112%	110%	129%
24	122%	120%	130%	126%

MIP-1 β				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	82	89	51	97
1:6	43	48	24	48
1:12	19	23	14	23
1:24	11	13	<LLOQ	12
6	104%	107%	95%	98%
12	93%	101%	110%	94%
24	109%	112%	<LLOQ	97%

TNF α				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	30	23	84	44
1:6	12	9.0	50	23
1:12	7.4	<LLOQ	27	13
1:24	<LLOQ	<LLOQ	13	<LLOQ
6	78%	79%	118%	104%
12	100%	<LLOQ	127%	116%
24	<LLOQ	<LLOQ	122%	<LLOQ

6. **Freeze/thaw stability:** Samples were assessed for freeze-thaw stability after 1, 2, and 3 F/T cycles. All values were within the acceptance range of 80-120% for freeze-thaw samples compared to the non-freeze thawed samples indicating that samples could be freeze-thawed up to 3 times without a loss in signal.

IFN α					
pg/mL		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	674	675	730	632
	FT-1X	638	626	690	593
	FT-2X	591	694	695	618
	FT-3X	653	685	709	614
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	95%	93%	95%	94%
	FT-2X	88%	103%	95%	98%
	FT-3X	97%	101%	97%	97%

IFN γ					
pg/mL		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	56	55	53	58
	2hr RT	53	50	56	57
	2hr 4C	50	54	54	58
	4hr 4C	55	54	52	57
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	93%	91%	105%	97%
	2hr 4C	89%	98%	101%	98%
	4hr 4C	97%	99%	98%	97%

IL-1 β					
pg/mL		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	268	217	125	159
	FT-1X	261	217	123	160
	FT-2X	250	219	121	157
	FT-3X	261	218	120	164
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	97%	100%	98%	101%
	FT-2X	93%	101%	96%	99%
	FT-3X	97%	100%	96%	103%

IL-6	
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	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	83	107	31	40
	FT-1X	82	102	30	39
	FT-2X	76	101	29	38
	FT-3X	80	105	28	38
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	98%	96%	94%	96%
	FT-2X	91%	95%	93%	95%
	FT-3X	96%	98%	90%	95%

	IL-8				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	116	113	10	27
	FT-1X	113	110	11	26
	FT-2X	108	110	12	26
	FT-3X	114	112	11	28
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	97%	98%	102%	98%
	FT-2X	93%	97%	110%	97%
	FT-3X	98%	100%	107%	106%

	IP-10				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	13101	14400	2960	2815
	FT-1X	13176	13050	3000	2840
	FT-2X	12968	14150	3005	2775
	FT-3X	13190	14350	3005	2715
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	101%	91%	101%	101%
	FT-2X	99%	98%	102%	99%
	FT-3X	101%	100%	102%	96%

	MDC				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	6.1	3.3	1.6	8.0
	FT-1X	5.1	3.2	1.7	7.4
	FT-2X	4.7	3.3	1.6	6.8
	FT-3X	5.6	3.7	1.6	7.8
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	84%	99%	109%	92%
	FT-2X	77%	100%	97%	84%
	FT-3X	92%	114%	102%	97%

	MIP-1 β
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	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	132	129	205	128
	FT-1X	130	128	205	126
	FT-2X	126	132	204	127
	FT-3X	128	129	203	127
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	99%	99%	100%	99%
	FT-2X	96%	102%	99%	100%
	FT-3X	97%	100%	99%	99%

	TNF α				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	120	86	105	73
	FT-1X	116	80	100	71
	FT-2X	106	85	90	72
	FT-3X	116	81	96	73
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	97%	92%	96%	97%
	FT-2X	89%	99%	85%	99%
	FT-3X	97%	94%	91%	100%

7. Bench Top Stability: Samples were assessed bench top stability and 2hr RT, and 2hr & 4hr 4°C to determine if the samples were stable on the bench prior to the assay or if refrigeration was required. All values were within the acceptance range of 80-120% for samples compared to the bench top samples indicating that no loss in activity will occur during the testing of the samples.

	IFN α				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	581	650	736	638
	2hr RT	650	719	699	583
	2hr 4C	659	707	733	644
	4hr 4C	700	722	740	655
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	112%	111%	95%	91%
	2hr 4C	114%	109%	100%	101%
	4hr 4C	120%	111%	100%	103%

		IFN γ			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	53	59	61	57
	2hr RT	60	59	57	55
	2hr 4C	60	60	58	61
	4hr 4C	59	60	59	63
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	113%	100%	92%	96%
	2hr 4C	112%	102%	95%	107%
	4hr 4C	111%	101%	95%	110%

		IL-1 β			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	248	232	132	172
	2hr RT	275	280	128	192
	2hr 4C	275	241	132	171
	4hr 4C	276	249	129	182
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	111%	120%	97%	112%
	2hr 4C	111%	104%	100%	100%
	4hr 4C	112%	107%	98%	106%

		IL-6			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	79	110	32	42
	2hr RT	76	105	29	37
	2hr 4C	83	110	30	39
	4hr 4C	79	107	29	40
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	96%	95%	93%	87%
	2hr 4C	104%	100%	95%	94%
	4hr 4C	100%	97%	90%	95%

		IL-8			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	113	117	14	31
	2hr RT	114	121	16	28
	2hr 4C	116	120	12	28
	4hr 4C	119	119	11	28
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	101%	103%	113%	91%
	2hr 4C	103%	102%	87%	92%
	4hr 4C	105%	101%	83%	92%

	IP-10				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	12979	15700	2995	2770
	2hr RT	13183	16800	3050	2690
	2hr 4C	13751	15900	3040	2905
	4hr 4C	13630	17300	3075	2935
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	102%	107%	102%	97%
	2hr 4C	106%	101%	102%	105%
	4hr 4C	105%	110%	103%	106%

	MDC				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	5.9	4.4	2.2	8.9
	2hr RT	6.1	4.4	2.0	7.2
	2hr 4C	6.4	4.2	1.8	8.2
	4hr 4C	5.8	4.5	1.9	8.5
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	104%	100%	91%	80%
	2hr 4C	109%	95%	82%	93%
	4hr 4C	99%	100%	88%	96%

	MIP-1β				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	127	137	210	129
	2hr RT	133	140	206	125
	2hr 4C	134	133	205	130
	4hr 4C	140	134	212	131
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	105%	103%	98%	97%
	2hr 4C	106%	97%	98%	101%
	4hr 4C	111%	98%	101%	102%

	TNFα				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	108	90	106	81
	2hr RT	115	91	104	69
	2hr 4C	121	91	102	77
	4hr 4C	118	86	98	70
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	106%	102%	98%	85%
	2hr 4C	111%	102%	96%	95%
	4hr 4C	109%	96%	93%	87%









Porcine Cytokine Panel 1 Validation Report V1

Final Audit Report

2025-06-09

Created:	2025-06-09
By:	Laurie Stephen (lstephen@ampersandbio.com)
Status:	Signed
Transaction ID:	CBJCHBCAABAARbM0xgGT8S6lh1SCveA85aqcSDJEywac

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