

Supplementary Materials: Quality-Related Properties of Equine Immunoglobulins Purified by Different Approaches

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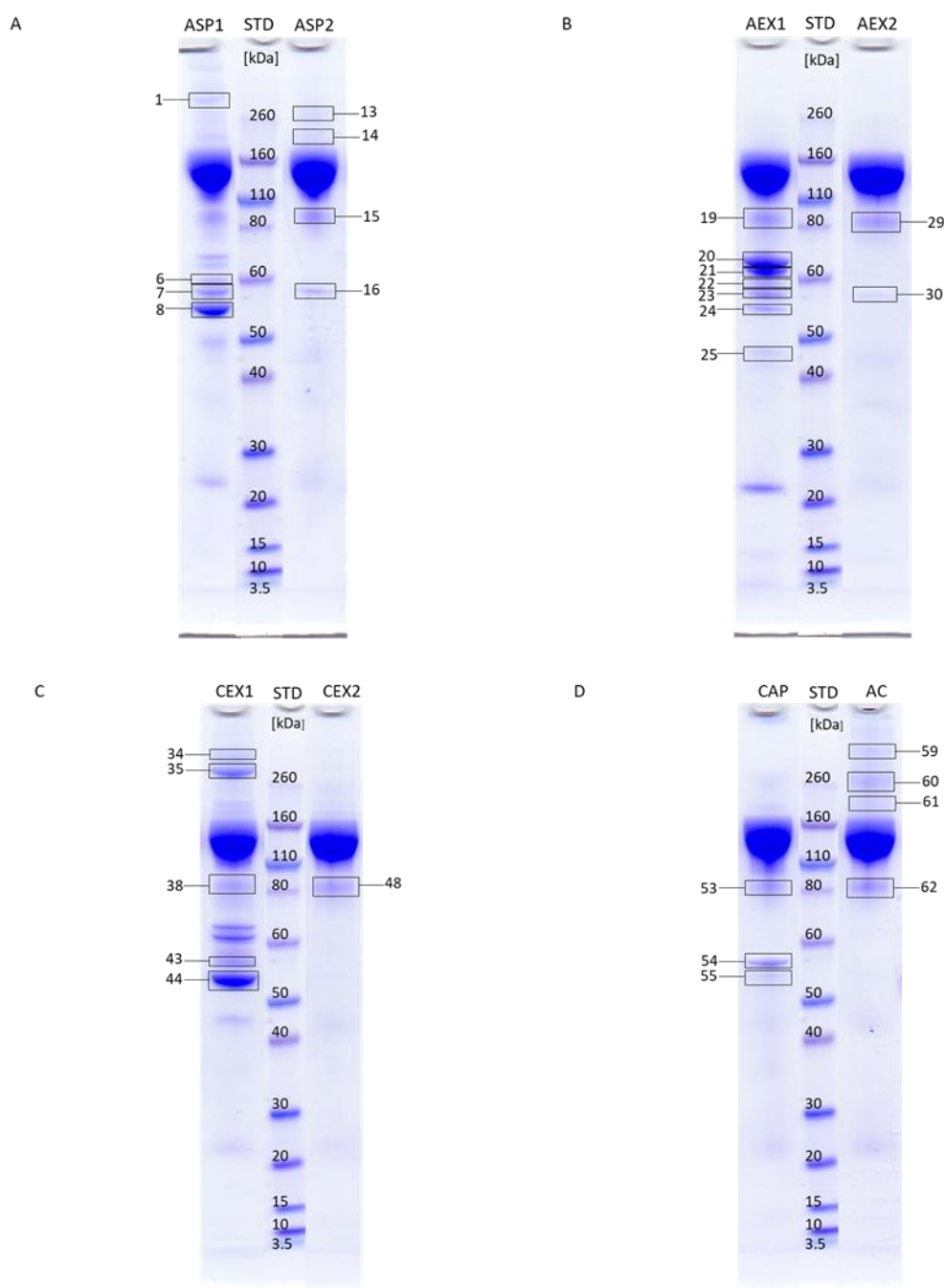


Figure S1. SDS-PAGE analysis of purified IgG fractions under non-reducing conditions with annotation of protein bands identified by MS/MS analysis (in Table S1). IgG samples were obtained by ASP (A), AEX (B), CEX (C), CAP (D) and AC (D) refinement protocols (same samples as in Figure 3). Other protein bands remained unidentified.

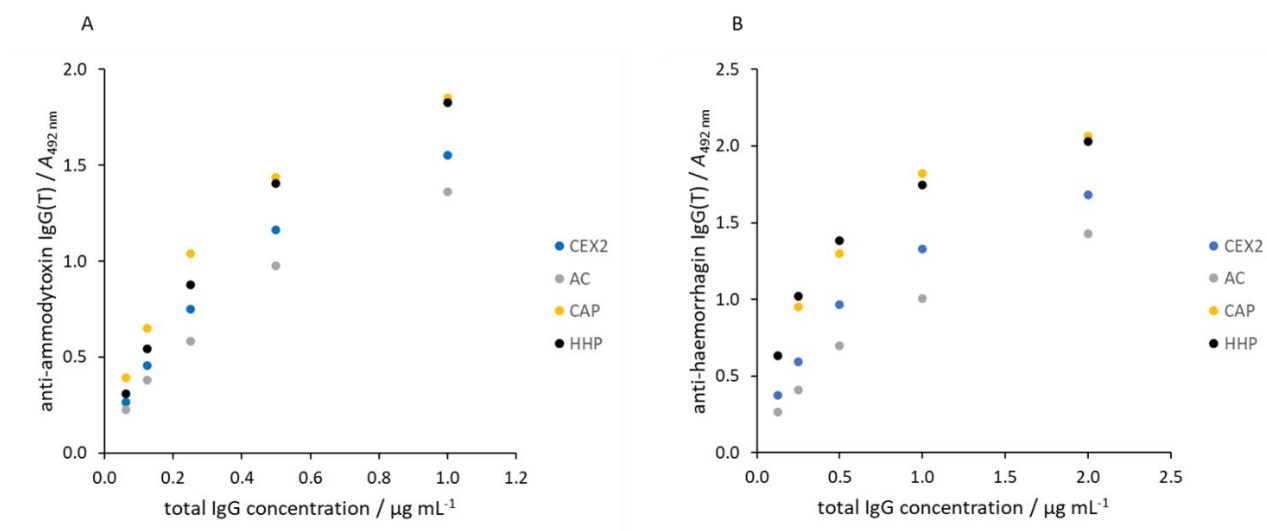


Figure S2. Ammodytotoxin- and haemorrhagin-specific IgG(T) subclass determination in purified IgG fractions. Ammodytotoxin (Atx) (A)- and haemorrhagin (H)-specific IgG(T) subclass (B) were determined in protein A affinity chromatography (AC), cation-exchange chromatography (CEX2) and caprylic acid precipitation (CAP) samples in comparison to hyperimmune horse plasma (HHP) determined by ELISA. Atx- or H-coated microtiter plates were incubated with IgG preparations and HHP prepared in equal total IgG concentration. Anti- IgG(T) conjugated with HRP was added to visualise Atx- and H- specific antibodies of only IgG(T) subclass.

Table S1. List of proteins identified by MS/MS analysis in the IgG preparations after ASP, AEX, CEX, CAP and AC purification processing.

Sample Lane	Gel Piece	[M+H] ⁺ Peptide	Protein Name	Protein ID	Protein Score	Peptide Sequence
ASP1	1	1775.98	immunoglobulin gamma 5 heavy chain constant region ,partial	CAC86340.1	82	R.VPQVYVLAPHPDELAK.N
		1806.04	immunoglobulin gamma 5 heavy chain constant region ,partial	CAC86340.1	54	R.VVSVLP IQHQDWLSGK.E
			immunoglobulin gamma 4 heavy chain, partial	AAS18415.1		
			immunoglobulin gamma 2 heavy chain constant region, partial	CAC44761.1		
	6		immunoglobulin gamma 4 heavy chain constant region, partial	CAC44762.1		
		1992.98	histidine-rich glycoprotein	XP_023479252.1	67	K.DSPVLFDFFEDTELYR.Q
		2048.99			105	R.VSFHLDALAPGDGGLYTCR.Y
		2854.40	alpha-1B-glycoprotein	XP_023507318.1	105	R.REGDDQFLEVAEAPEDVVVTFPV HR.A
ASP2	8	2319.18	serum albumin precursor	NP_001075972.1	105	K.LPESERLPCSENHLALALNR.L
	13	2335.12	immunoglobulin alpha constant heavy chain, partial	AAP80145.1	62	R.LSGKPTHVNVSVVMAEADGTCY
		2642.18	immunoglobulin gamma 5 heavy chain constant region, partial	CAC86340.1	109	K.DFYPP EIDVEWQSNEHPEPEGK. Y
	14	1541.73	immunoglobulin gamma 4 heavy chain constant region, partial	CAC44762.1	63	K.DFYPTDIDIEWK.S
			immunoglobulin gamma	CAC44763.1		

AEX1	15	2642.20	7 heavy chain constant region, partial immunoglobulin gamma 5 heavy chain constant region, partial	CAC86340.1	123	K.DFYPPPEIDVIEWQSNEHPEPEGK.Y
		1763.87	immunoglobulin gamma 4 heavy chain constant region, partial immunoglobulin gamma 7 heavy chain constant region, partial	CAC44762.1 CAC44763.1	98	K.SQTYICNVAHPASSTK.V
			immunoglobulin gamma 6 heavy chain constant region, partial	CAC86341.1.1		
		2646.22	immunoglobulin gamma 5 heavy chain constant region, partial	CAC86340.1	80	K.DFYPPPEIDVIEWQSNEHPEPEGK.Y
		2048.99			63	R.VSFHLDALAPGDGGLYTCR.Y
	16	2698.32	alpha-1B-glycoprotein	XP_023507318.1	155	R.EGDDQFLEVAEAPEDVVVTFPV HR.A
		2747.46			105	R.THAAGDPSGPSAPVTIEELAAPP PPVLR.V
	19	1541.73	immunoglobulin gamma 4 heavy chain constant region, partial immunoglobulin gamma 7 heavy chain constant region, partial	CAC44762.1 CAC44763.1	84	K.DFYPTDIDIEWK.S
		2143.01	immunoglobulin gamma 5 heavy chain constant region, partial immunoglobulin gamma 4 heavy chain constant	CAC86340.1 CAC44762.1	110	K.YSTTPAQLNSDGSYFLYSK.L

		region, partial immunoglobulin gamma 7 heavy chain constant region, partial	CAC44763.1		
	1994.83	serotransferrin precursor	NP_001075415.2	78	K.CACSNHEPYFGYSGAFK.C
20	2686.32	transferrin, partial	AAF05482.1	79	K.HSTVLENLPQEADRDEYQLLCR. D
	2714.37			94	R.SVDGKEDLIWGLLNQAQEHFGT EK.S
21	1097.50	serotransferrin precursor	NP_001075415.2	58	R.YYGYTGAFR.C
	2301.10	transferrin, partial	AAC78365.1	101	K.AVTEFESCNLAEAPNHAVVSR.K
22	1178.56	histidine-rich	XP_023479252.1	63	R.HFGHPFHSGR.H
	1992.92	glycoprotein		157	K.DSPVLDFDFEDELTYR.Q
23	2698.33	alpha-1B-glycoprotein	XP_023507318.1	149	R.EGDDQFLEVAEAPEDVVVTFPV HR.A
24	1932.05	serum albumin precursor	NP_001075972.1	60	K.SLHTLFGDKLCTVATLR.A
	2319.29			62	K.LPESERLPCSENHLALALNR.L
	2431.15			124	K.NCDLFEEVGEYDFQNALIVR.Y
25	2291.08	beta-2-glycoprotein 1	XP_001499728.2	59	K.TSYVPGEIIVYSCQPGYVSR.G
AEX2	1763.82	immunoglobulin gamma 4 heavy chain constant region, partial	CAC44762.1	100	K.SQTYICNVAHPASSTK.V
29		immunoglobulin gamma 7 heavy chain constant region, partial	CAC44763.1		
		immunoglobulin gamma 6 heavy chain constant region, partial	CAC86341.1		
	1794.02	immunoglobulin gamma 7 heavy chain constant region, partial	CAC44763.1	84	R.VVSILAIQHKDWLSGK.E
	1805.98	immunoglobulin gamma	CAC44762.1	72	R.VVSVLPQHKDWLSGK.E

			4 heavy chain constant region, partial immunoglobulin gamma 2 heavy chain constant region, partial immunoglobulin gamma 5 heavy chain constant region, partial	CAC44761.1		
				CAC86340.1		
CEX1	34	3049.59	predicted: alpha-2-macroglobulin-like	XP_008534363.1	83	R.EVLESLTEEAVKEDNSVHWTRPQ KPK.A
		1654.89			88	R.AEHPFIVEEFVLPK.F
	35	1828.91			108	K.QLTFPLSSEPFQGSYK.V
		2518.08	predicted: alpha-2-macroglobulin-like	XP_008534363.1	160	K.VYDYYETDEFAIAEYNAPCGK.D
		3049.58			98	R.EVLESLTEEAVKEDNSVHWTRPQ KPK.A
	38	2037.07	immunoglobulin gamma 1 heavy chain constant region, partial	CAC44624.1	63	R.SQEPQVYVLAPHPDELSK.S
		2920.40	immunoglobulin gamma 7 heavy chain, partial	AAS18414.1	64	K.ECGGCPTCPECLSVGPSVFIFPPK PK.D
	43	1914.03			91	R.FNPVSGEVPPKYPLDVR.D
		2752.37	hemopexin	XP_005612174.1	111	K.SGAAATWTELPWPHEKVDGAL CVEK.S
	44	2319.15			76	K.LPESERLPCSENHLALALNR.L
		2431.09	serum albumin precursor	NP_001075972.1	178	K.NCDLFEEVGEYDFQNALIVR.Y
		2559.19			175	K.K NCDLFEEVGEYDFQNALIVR.Y
CEX2	48	1763.83	immunoglobulin gamma 4 heavy chain constant region, partial immunoglobulin gamma 7 heavy chain constant region, partial	CAC44762.1	82	K.SQTYICNVAHPASSTK.V
				CAC44763.1		

		immunoglobulin gamma 6 heavy chain constant region, partial	CAC86341.1		
CAP	1804.61	immunoglobulin gamma 4 heavy chain constant region, partial	CAC44762.1	56	R.TISKPTGQPR.E
		immunoglobulin gamma 7 heavy chain constant region, partial	CAC44763.1		K.TISKPTGQPR.E
53	1763.84	immunoglobulin gamma 4 heavy chain constant region, partial	CAC44762.1	85	K.SQTYICNVAHPASSTK.V
		immunoglobulin gamma 7 heavy chain constant region, partial	CAC44763.1		
		immunoglobulin gamma 6 heavy chain constant region, partial	CAC86341.1		
	2037.04	immunoglobulin gamma 1 heavy chain constant region, partial	CAC44624.1	61	R.SQEPQVYVLAPHPDELSK.S
54	1114.61			76	R.ALWSGAVTPGR.D
	1877.99			84	R.HWADLVLSYVGPQHAGK.Y
	1935.14			59	R.VQEASAQVLRPGVSLRPK.C
	2747.47	alpha-1B-glycoprotein	XP_023507318.1	194	R.THAAGDPSPGAPVTIEELAAPP PPVLR.V
	3035.55			107	K.DGVAQEPVQVGSPTIEHQFPLGA VTS DTR.G
55	2431.15	serum albumin precursor	NP_001075972.1	89	K.NCDLFEEVGEYDFQNALIVR.Y
	2504.24	albumin, partial	AAG40944.1	106	K.LKPEPDAQCAAFQEDPDKFLGK. Y
	2504.24	serum albumin precursor	NP_001075972.1	105	K.LKPEPDAQCAAFQEDPDKFLGK. Y

AC	59	1378.75	immunoglobulin gamma	CAC44624.1	60	K.VNNQALPQPIER.T
		1615.83	1 heavy chain constant		83	R.IQHQDWLSGKEFK.C
		2188.96	region, partial		161	K.YSTTQAQQDSDGSYFLYSK.L
	60	1775.97	immunoglobulin gamma 5 heavy chain constant region, partial	CAC86340.1	88	R.VPQVYVLAPHPDELAK.N
		1378.77	immunoglobulin gamma		69	K.VNNQALPQPIER.T
		1615.85	1 heavy chain constant		74	R.IQHQDWLSGKEFK.C
		2037.07	region, partial		94	R.SQEPQVYVLAPHPDELSK.S
	61	2189.00	region, partial	CAC44624.1	129	K.YSTTQAQQDSDGSYFLYSK.L
		1378.76	immunoglobulin gamma		58	K.VNNQALPQPIER.T
		1615.85	1 heavy chain constant		72	R.IQHQDWLSGKEFK.C
	62	2037.06	region, partial	CAC44762.1	92	R.SQEPQVYVLAPHPDELSK.S
		1541.72	immunoglobulin gamma 4 heavy chain constant region, partial		75	K.DFYPTDIDIEWK.S
			immunoglobulin gamma 7 heavy chain constant region, partial		75	K.DFYPTDIDIEWK.S
		1880.99	immunoglobulin gamma 7 heavy chain, partial		61	R.EPQVYVLAPHRDELSK.N
			immunoglobulin gamma 4 heavy chain, partial		61	R.EPQVYVLAPHRDELSK.N
		2037.03	immunoglobulin gamma 1 heavy chain constant region, partial		65	R.SQEPQVYVLAPHPDELSK.S

Table S2. Summary of quality-related properties of IgGs purified by ammonium sulphate precipitation (ASP), anion-exchange chromatography (AEX), cation-exchange chromatography (CEX), caprylic acid precipitation (CAP) and protein A affinity chromatography (AC).

Purification Procedure	Purity (%)	Retained Impurities	Aggregate Content (%)	Melting Temperature of Pure IgG (°C)	Melting Temperature of Pure IgG + Sorbitol (°C)	IgG Subclass Distribution Shift (Factor of Increase (↑) / Decrease (↓))	Specific Activity [R^* / γ (IgG)]
ASP1	83.1 ± 1.5	histidine-rich glycoprotein alpha-1B-glycoprotein albumin	4.5 ± 1.4	-	-	IgGa ↑ 1.06 IgGb ↑ 1.33 IgG(T) ↓ 1.25	-
ASP2	96.6 ± 1.2	alpha-1B-glycoprotein	1.4 ± 0.2	70.21	76.82	-	-
AEX1	76.7 ± 0.8	transferrin histidine-rich glycoprotein alpha-1B-glycoprotein albumin beta-2-glycoprotein 1	0.0 ± 0.0	-	-	IgGa ↑ 1.51 IgGb ↑ 1.54 IgG(T) ↓ 2.02	-
AEX2	98.0 ± 0.8	alpha-1B-glycoprotein	0.4 ± 0.1	70.38	76.51	-	-
CEX1	72.6 ± 2.2	alpha-2-macroglobulin-like transferrin hemopexin albumin	5.4 ± 0.9	-	-	IgGa ↑ 1.42 IgGb ↑ 2.44 IgG(T) ↓ 2.65	-

CEX2	97.8 ± 0.8	-	1.2 ± 0.3	70.60	76.08	-	0.45
CAP	93.3 ± 1.4	alpha-1B-glycoprotein albumin	2.3 ± 0.3	69.69	74.98	IgGa ↓ 1.52	1.07
						IgGb ↓ 1.17	
						IgG(T) ↓ 1.11	
AC	92.1 ± 1.7	-	6.4 ± 1.4	68.54	73.4	IgGa ↑ 10.06	<0.41
						IgGb ↑ 2.55	
						IgG(T) ↓ 3.96	

* neutralisation potency expressing the number of LD₅₀ doses that can be neutralised by 1 mL of pure IgG preparation.