

C. Howe, C. Goebel, G. Trout and R. Kazlauskas

Equine Growth Hormone

Australian Sports Drug Testing Laboratory (ASDTL), National Measurement Institute, 1 Suakin St., Pymble, NSW 2073 Australia.

Background

During routine cleaning of athletes' rooms at an Australian Institute of Sport facility at Del Monte, South Australia, a bucket and a plastic bag with injection materials was found. Within this bucket were vials with part labels. This resulted in an accusation of "group injecting sessions" by an athlete who also denied knowledge of the vials. The incident was investigated by the Australian Sports Commission and Cycling Australia. Further, an investigation into this incident was carried out by Justice Anderson in June 2004 (Anderson 2004).

Experimental

Vial contents

The involvement of ASDTL started when the vials were sent to us in December 2003. In total 13 vials were received, of which 9 had no label and 4 had a partial label. It was possible to deduce that they originated from Commonwealth Serum Laboratories and the contents were possibly Equigen, which was an equine growth hormone (eGH) preparation.

One vial still had enough liquid in it to analyse for eGH. LC-ESI-MS gave a molecular weight of 21887Da and the Swiss-Prot entry P01245 (<http://au.expasy.org/sprot/>) gives equine growth hormone as 21757Da. With the addition of a methionine the molecular weight will be expected at 21888Da which is in agreement with the value measured. When authentic material obtained from Bresagen (the manufacturers of the eGH) was measured it provided a molecular ion at 21887Da corresponding to methionine equine growth hormone. The contents of all the other vials gave same result.



Figure 1 The four vials which had some of the labels intact.

Peptide mapping after trypsin digestion was analysed by LC-ESI-MS/MS at ASDTL using routine procedures (Goebel, 2005). Both the standard material and vial samples matched for fragments T3 (Amino acids 30-33), T7 (70-76), T8 (77-94), T9 (95-107), T10 (108-111), T12 (114-124) and T21 (171-176).

To further confirm the identity the sample was sent as an unknown peptide (but information that the origin was from horse was provided) to the Australian Proteome Analysis Facility at Macquarie University. They performed peptide mapping with trypsin and MALDI-TOF-TOF and they confirmed the peptide as equine growth hormone and also were able to show the presence of the N-terminal methionine residue.

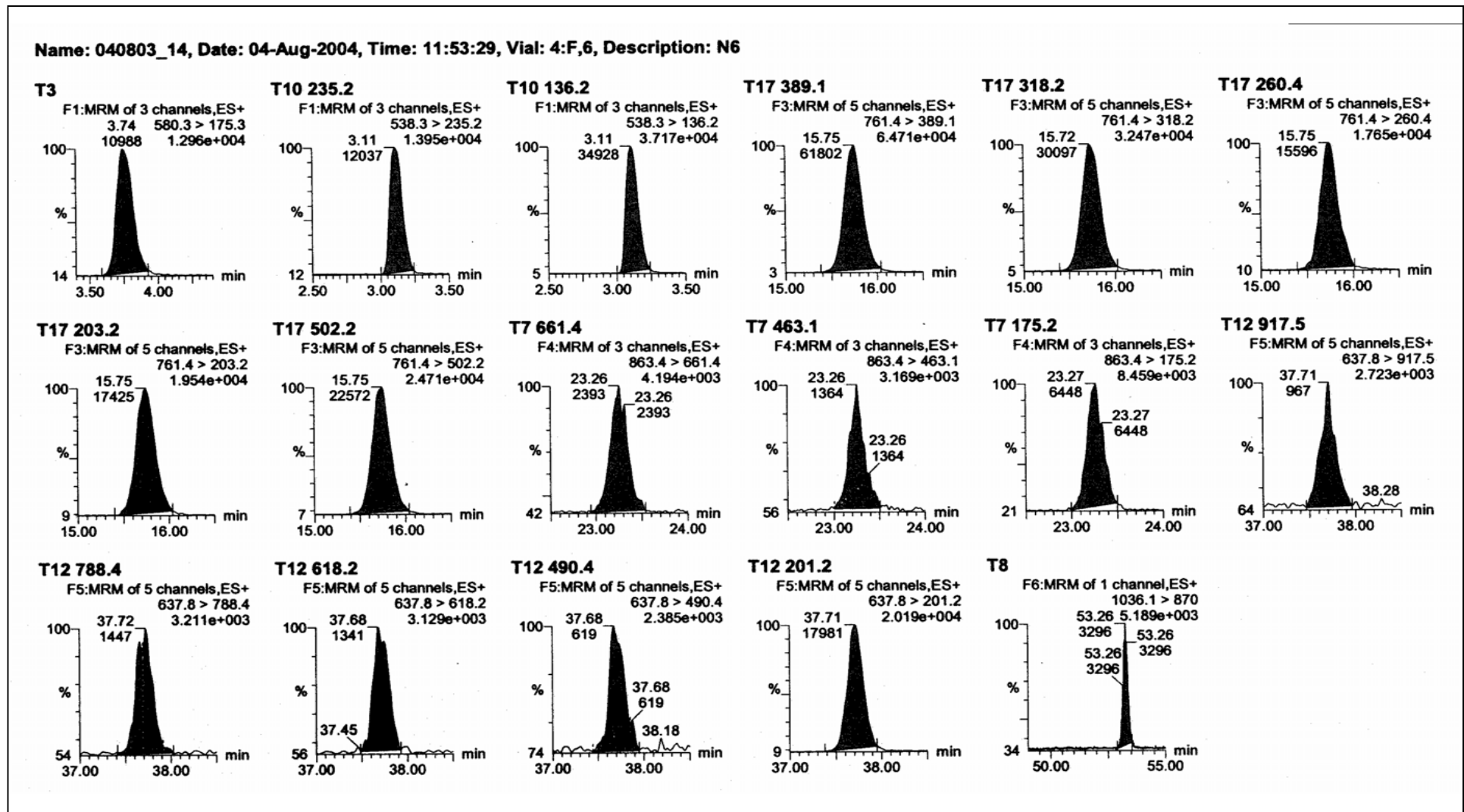


Figure 2 The LC-ESI-MSMS printout for the tryptic digest of the eGH for characteristic ions for each of the fragments.

The comparative sequence of equine and human growth hormone are shown in **Figure 3**. The direct overlap of amino acids is small and only amounts to 18.8% when based on the raw sequence.

Human		F	P	T	I	P	L	S	R	L	F	D	N	A	M	L	R	A	H	R	L	H	Q	L	A	F	D	T	Y	Q	E
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Equine (M)		F	P	A	M	P	L	S	S	L	F	A	N	A	V	L	R	A	Q	H	L	H	Q	L	A	A	D	T	Y	K	E
Human		F	E	E	A	Y	I	P	K	E	Q	K	Y	S	F	L	Q	N	P	Q	T	S	L	C	F	S	E	S	I	P	T
		31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Equine		F	E	R	A	Y	I	P	E	G	Q	R	Y	S	I	Q	N	A	Q	A	A	F	C	F	S	E	T	I	P	A	P
Human		P	S	N	R	E	E	T	Q	Q	K	S	N	L	E	L	L	R	I	S	L	L	L	I	Q	S	W	L	E	P	V
		61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
Equine		T	G	K	D	E	A	Q	Q	R	S	D	M	E	L	L	R	F	S	L	L	L	I	Q	S	W	L	G	P	V	Q
Human		Q	F	L	R	S	V	F	A	N	S	L	V	Y	G	A	S	D	S	N	V	Y	D	L	L	K	D	L	E	E	G
		91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
Equine		L	L	S	R	V	F	T	N	S	L	V	F	G	T	S	D	R	V	Y	E	K	L	R	D	L	E	E	G	I	Q
Human		I	Q	T	L	M	G	R	L	E	D	G	S	P	R	T	G	Q	I	F	K	Q	T	Y	S	K	F	D	T	N	S
		121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
Equine		A	L	M	R	E	L	E	D	G	S	P	R	A	G	Q	I	L	K	Q	T	Y	D	K	F	D	T	N	L	R	S
Human		H	N	D	D	A	L	L	K	N	Y	G	L	L	Y	C	F	R	K	D	M	D	K	V	E	T	F	L	R	I	V
		151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
Equine		D	D	A	L	L	K	N	Y	G	L	L	S	C	F	K	K	D	L	H	K	A	E	T	Y	L	R	V	M	K	C
Human		Q	C	R	S	V	E	G	S	C	G	F																			
		181	182	183	184	185	186	187	188	189	190	191																			
Equine		R	R	F	V	E	S	S	C	A	F																				

18.8% match on raw sequences

Figure 3 the comparative sequence of hGH and eGH showing overlap of only 18.8% of the amino acids. The numbered boxes that are shaded represent overlap.

However the equine growth hormone appears to have at least 2 deletions from the hGH and conversely the hGH appears to have one deletion compared to eGH. When the sequences are re-aligned to take these into account the match is increased to 66.3% showing many contiguous amino acid sequences (**Figure 4**).

Human		F	P	T	I	P	L	S	R	L	F	D	N	A	M	L	R	A	H	R	L	H	Q	L	A	F	D	T	Y	Q	E
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Equine (M)		F	P	A	M	P	L	S	S	L	F	A	N	A	V	L	R	A	Q	H	L	H	Q	L	A	A	D	T	Y	K	E
Human		F	E	E	A	Y	I	P	K	E	Q	K	Y	S	F	L	Q	N	P	Q	T	S	L	C	F	S	E	S	I	P	T
		31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Equine		F	E	R	A	Y	I	P	E	G	Q	R	Y	S	-	I	Q	N	A	Q	A	A	F	C	F	S	E	T	I	P	A
Human		P	S	N	R	E	E	T	Q	Q	K	S	N	L	E	L	L	R	I	S	L	L	L	I	Q	S	W	L	E	P	V
		61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
Equine		P	T	G	K	D	E	A	Q	Q	R	S	D	M	E	L	L	R	F	S	L	L	L	I	Q	S	W	L	G	P	V
Human		Q	F	L	R	S	V	F	A	N	S	L	V	Y	G	A	S	D	S	N	V	Y	D	L	L	K	D	L	E	E	G
		91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
Equine		Q	L	L	S	R	V	F	T	N	S	L	V	F	G	T	S	D	R	-	V	Y	E	K	L	R	D	L	E	E	G
Human		I	Q	T	L	M	G	R	L	E	D	G	S	P	R	T	G	Q	I	F	K	Q	T	Y	S	K	F	D	T	N	S
		121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
Equine		I	Q	A	L	M	R	E	L	E	D	G	S	P	R	A	G	Q	I	L	K	Q	T	Y	D	K	F	D	T	N	L
Human		H	N	D	D	A	L	L	K	N	Y	G	L	L	Y	C	F	R	K	D	M	D	K	V	E	T	F	L	R	I	V
		151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
Equine		R	S	D	D	A	L	L	K	N	Y	G	L	L	S	C	F	K	K	D	L	H	K	A	E	T	Y	L	R	V	M
Human		Q	C	R	S	-	V	E	G	S	C	G	F																		
		181	182	183	184	185	186	187	188	189	190	191	192																		
Equine		K	C	R	R	F	V	E	S	S	C	A	F																		
													66.3% match on adjusted sequences																		

Figure 4 The realigned comparative sequence for eGH and hGH taking into account deletions. The numbered boxes that are shaded represent overlap.

Efficacy

Attempts have been made to use domestic animal GH for children with short stature but only higher primate GH works in humans (Behncken, 1997; Souza, 1995). The main concerns with use of eGH can be proposed to be the development of antibodies and development of sensitivity giving allergic reactions. Further exposure to counterfeits and poorly made preparations will occur and several such preparations have been seized by Australian Customs Service and analysed at ASDTL. These also could potentially give rise to sensitisation and allergic reactions.

Acknowledgements

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References

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