

Patient number	Hb var true ID	Hb experimental ID*	Correct ID frequencies and Replicates results
IH140064	HbSS	HbS	<u>9/10 CORRECT tentative HbS ID</u> <u>Breakdown:</u> 5/10 samples: correct ID, all 3 replicates in agreement 3/10 samples: correct ID, 2/3 replicates in agreement 1/10 samples: correct ID, 1/3 replicates show HbC 1/10 samples: inconclusive as HbS indicated only by one replicate together with another variant
IH140066	HbS	HbS	
IH140067	HbS+HPFH#	HbS	
IH140069	HbS	HbS	
IH140081	HbS	inconclusive	
IH140086	HbS	HbS	
IH087370#	HbS	HbS	
IH140464*	HbS	HbS	
IH140471*	HbS	HbS	
IH140466*	HbS	HbS	
IH140065	HbAC	inconclusive	<u>8/11 CORRECT tentative HbC ID</u> <u>Breakdown:</u> 4/11 samples: correct ID, all 3 replicates in agreement 3/11 samples: correct ID, 2/3 replicate show HbC 1/11 samples: correct ID, 1/3 show HbC, 2/3 show no variant 1/11 sample: incorrect ID, all 3 replicates show no HbC proteotypic peptides 2/11 samples: inconclusive as HbC is indicated together with other variants
IH140068	HbAC	inconclusive	
IH140073	HbAC	HbC	
IH140076	HbAC	HbC	
IH140078	HbAC	HbC	
IH140080	HbAC	HbC	
IH140081	HbAC	HbC	
IH140084	HbAC	HbC	
IH087364#	HbAC	No variants	
IH140473*	HbAC	HbC	
IH140486*	HbAC	HbC	
IH140488*	HbAC	HbC	
IH140071	HbD-Punjab/Los Angeles	No variants	<u>1/7 samples correct tentative HbD Punjab/Los Angeles ID</u> <u>Breakdown:</u> 1/7 samples: correct ID, all 3 replicates in agreement 1/7 samples: inconclusive, replicates show concomitantly different variants 5/7 samples: incorrect, no variants ID, all 3 replicates show no HbD Punjab Los Angeles proteotypic peptides.
IH140072	HbD-Punjab/Los Angeles	inconclusive	
IH140077	HbD-Punjab/Los Angeles	HbD Punjab/Los Angeles	
IH140083	HbD-Punjab/Los Angeles	No variants	
IH140485*	HbD-Punjab/Los Angeles	No variants	
IH140467*	HbD-Punjab/Los Angeles	No variants	
IH087372#	HbD-Punjab/Los Angeles	No variants	
IH140074	HbEE	HbE	<u>6/10 samples CORRECT tentative HbE ID</u> <u>Breakdown:</u> 5/10 samples: correct ID, all 3 replicates in agreement 1/10 samples: correct ID, 2/3 replicates indicate HbE proteotypic peptides. 3/10 samples: incorrect, no variants ID, all 3 replicates in agreement
IH140075	HbAE	HbE	
IH140079	HbAE	HbE	
IH140082	HbAE	HbE	
IH087362#	HbAE	No variants	
IH087365#	HbAE	No variants	
IH140470*	HbAE	HbE or HbD-Iran	
IH140465*	HbAE	HbE	

IH140489*	HbAE	HbE	1/10 samples: inconclusive, uncertainty between HbE and HbD Iran
IH140491*	HbAE	HbE	
IH140484*	HbD-Iran	HbD-Iran	<u>1/1 samples CORRECT tentative HbD Iran ID Breakdown:</u> 1/1 samples correct ID, all 3 replicates in agreement
IH140468*	HbJ-Baltimore	No variant	<u>1/1 sample missed HbJ detection</u> <u>Breakdown:</u> (no HbJ Baltimore proteotypic peptides detected)
IH140490*	HbA0	No variant	<u>3/3 correct ID:</u> no Hb var proteotypic peptides detected. All replicates in agreement
IH087366#	HbA0	No variant	
IH087367#	HbA0	No variant	

Table S1. MALDI MS putative identifications of Hb var from patients' blood samples conducted on the MALDI qTOF Synapt G2 HDMS. A column is added on the technical replicates result to explain how the "claim" on the Hb var identity was reached. The patients' number labelled with (*) indicate the batch received on 25.11.2020. The patients number labelled with (#) indicate samples received on 28.11.2018. The patients' numbers not labelled with any symbol indicate the batch received on 10/10/2020. Note that the experimental ID as reported does not indicate the presence of a trait or of the pathology.

Patient no. and replicate	variant and peptide slice	Experimental <i>m/z</i> (Th)	Mass Accuracy (ppm)	Experimental ID	True ID
IH140064_3	HbS (2-9)	922.538	2.8	HbS	HbSS
IH140066_1	HbS (2-9)	922.527	9.7	HbS	HbAS
IH140067_1	HbS (2-9)	922.54	4.8	HbS	HbSS
IH140069_2	HbS (2-9)	922.537	1.5	HbS	HbAS
IH140081_1	HbS (2-9)	922.547	12.1	Inconclusive	HbAS
	HbE (19-31)	1313.705	-9.3		
IH087370_1	HbS (2-9)	922.536	-5.9	HbS	HbAS
IH140464_3	HbS (2-9)	922.535	-0.8	HbS	HbAS
IH140471_2	HbS (2-9)	922.538	2.3	HbS	HbAS
IH140487_1	HbS (2-9)	922.542	7.1	HbS	HbAS
IH140086_2	HbS (2-9)	922.537	1.4	HbS	HbAS
IH140473_2	HbC (2-9)	951.571	9	HbC	HbAC
IH140486_1	HbC (2-9)	951.567	4.9	HbC	HbAC
IH140488_1	HbC (2-9)	951.562	0.2	HbC	HbAC
	HbJ-Baltimore 10-31	2286.151	-9.3		
IH140073_2	HbC (2-9)	951.533	-9.4	HbC	HbAC
IH140076_3	HbC (2-9)	951.562	0.3	HbC	HbAC
IH140078_1	HbC (2-9)	951.564	1.7	HbC	HbAC
IH140080_1	HbC (2-9)	951.562	-0.2	HbC	HbAC
IH140084_3	HbC (2-9)	951.563	1.1	HbC	HbAC
IH087364_1	NA	NA	NA	no variants	HbAC
IH140065_3	HbC (2-9)	951.555	-8	Inconclusive	HbAC
	HbD-Iran (19-31)	1313.674	-5.4		
IH140068_1	HbC (2-9)	951.562	0.1	Inconclusive	HABC
IH140484_1	HbD-Iran 10-31	2227.183	0.2	HbD Iran	HbD-Iran HbAD- Punjab/Los Angeles
IH087372_1	NA	NA	NA	no variants	
IH140071_1	NA	NA	NA	no variants	HbAD- Punjab/Los Angeles
IH140072_2	HbD-Iran (19-31)	1313.671	-11.1	Inconclusive	HbAD- Punjab/Los Angeles
	HbE (19-31)	1313.703	-7.6		
IH140077_1	HbD-Punjab/Los Angeles (121- 133)	1377.705	-8.1	HbD- Punjab/Los Angeles	HbAD- Punjab/Los Angeles

IH140083_1	NA	NA	NA	no variants	HbAD Punjab/Los Angeles
IH140485_1	NA	NA	NA	no variants	HbAD Punjab/Los Angeles
IH140467_1	NA	NA	NA	no variants	HbAD- Punjab/Los Angeles
IH140470_2	HbE 10-27	1829.983	-4	HbE or HbD- Iran	HbAE
	HbE 10-31	2227.209	-4.6		
	HbD-Iran 10-31	2227.209	11.8		
IH140465_1	NA	NA	NA	no variants	HbAE
IH087362_1	NA	NA	NA	no variants	HbAE
IH087365_1	NA	NA	NA	no variants	HbAE
IH140489_1	HbE (10-31)	2227.226	3.1	HbE	HbAE
IH140491_3	HbE (10-27)	1829.964	-1.7		
	HbE (10-31)	2227.201	-8.1		
	HbD-Iran 10-31	2227.201	8.2		
IH14074_2	HbE (19-31)	1313.713	-3.5	HbE	HbEE
	HbE (10-31)	2227.237	8		
IH14075_2	HbE (19-27)	916.475	1.3	HbE	HbEE
	HbE (19-31)	1313.714	-2.7		
	HbE (10-27)	1829.985	5.2		
	HbE (10-31)	2227.239	9		
IH14079_1	HbE (19-31)	1313.706	-8.6	HbE	HbAE
	HbE (10-31)	2227.211	-3.9		
IH14082_2	HbE (19-31)	1313.713	-3.1	HbE	HbAE

Table S2. MALDI Synapt G2 HDMS m/z assignments to Hb var. HbJ Baltimore and HbA⁰ are not reported as no proteotypic peptides had been detected in both cases.

Sample 843	theoretical m/z 2286.1726	ID HBJ Baltimore (10-31)			
Ion fragm.	sequence	m/z (ttfLeX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)
MH+	SAVTALWDKVVNVDEVGGEALGR	2286.171	-0.8	2286.159	-5.8
b10	.SAVTALWDKV.n	1071.583	0.2	1071.579	-3.8
b11	.SAVTALWDKVN.v	1185.625	-1.0	--	--
b18	.SAVTALWDKVVNVDEVGGE.a	1870.917	-0.8	1870.912	-14.0
b21	.SAVTALWDKVVNVDEVGGEALG.r	--	--	2112.065	1.8
y1	g.R.	175.119	-1.2	--	--
y4	e.ALGR.	416.260	-4.8	416.261	-1.4
y6	g.GEALGR.	602.324	-2.0	602.324	-3.2
y7	v.GGEALGR.	659.345	-3.3	659.350	3.9
y8	e.VGGEALGR.	758.414	-1.9	758.408	-9.7
y9	d.EVGGEALGR.	887.457	-1.6	887.456	-2.6
y10	v.DEVGGEALGR.	1002.486	1.4	1002.474	-10.9
Y11	v.VDEVGGEALGR.	--	--	1101.535	-17.0
y12	v.NVDEVGGEALGR.	1215.601	3.6	1215.588	-6.8
y13	k.VNVDEVGGEALGR.	1314.666	0.6	1314.668	1.2
y14	d.KVVNVDEVGGEALGR.	1442.757	-2.0	1442.752	-5.2
y15	w.DKVVNVDEVGGEALGR.	1557.783	-2.1	1557.781	-4.4
Sample 844	theoretical m/z 694.4251	ID HBC (2-7)			
Ion fragm.	sequence	m/z (ttfLeX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)
b2	.VH.l	237.135	-2.8	--	--
b3	.VHL.t	350.219	-1.3	--	--
b4	.VHLT.p	451.266	-2.3	--	--
b5	.VHLTP.k	548.319	-3.3	--	--
y1	e.K			--	--
y3	l.TPK.	345.213	-4.9	--	--
y4	h.LTPK.	458.297	-2	--	--
y5	v.HLTPK.	595.356	-1	--	--
a2	.VH.l	209.14	-4	--	--
a3	.VHL.t	322.224	-1.8	--	--
Sample 844	theoretical m/z 951.5626	ID HBC (2-9)			
Ion fragm.	sequence	m/z (ttfLeX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)
MH+	VHLTPKEK	--	--	951.561	-1.5
b3	.VHL.t	--	--	350.218	-2.2
y1	e.K	--	--	147.114	-7.4
Sample 846	theoretical m/z 1313.717	ID HBC (19-31)			
Ion fragm.	sequence	m/z (ttfLeX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)

MH+	VNVDEVGGKALGR	--	--	1313.709	-5.9
b3	.VNV.d	--	--	313.388	4.0
b5	.VNVDE.v	--	--	413.267	13.9
y4	k.ALGR.	--	--	557.264	13.9
Sample 846	theoretical m/z 1829.976	ID HBE (10-27)			
Ion fragm.	sequence	m/z (ttfleX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)
MH+	SAVTALWGKVNDEVGGK	1829.970	3.3	--	--
b4	.SAVT.a	359.193	0.1	--	--
b6	.SAVTAL.w	543.314	-4.2	--	--
b7	.SAVTALW.g	729.393	-4.7	--	--
b9	.SAVTALWGK.v	914.509	-0.3	--	--
b10	.SAVTALWGKV.n	1013.578	-3.8	--	--
b11	.SAVTALWGKVN.v	1127.621	-1.7	--	--
b14	.SAVTALWGKVNDE.v	1470.759	-3.7	--	--
b15	.SAVTALWGKVNDEV.g	1569.827	1.5	--	--
b17	.SAVTALWGKVNDEVGG.k	1683.87	4.2	--	--
y10	g.KVNDEVGGK.	1044.568	1.2	--	--
y13	a.LWGKVNDEVGGK.	1400.753	-2.5	--	--
y15	v.TALWGKVNDEVGGK.	1572.838	2.3	--	--
Sample 847	theoretical m/z 1829.976	ID HBE (10-27)			
Ion fragm.	sequence	m/z (ttfleX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)
MH+	SAVTALWGKVNDEVGGK	1829.976	0.1	--	--
a10	.SAVTALWGKV.n	985.586	3.5	--	--
b10	.SAVTALWGKV.n	1013.578	0.1	--	--
b11	.SAVTALWGKVN.v	1127.619	-1.8	--	--
b12	.SAVTALWGKVVN.d	1226.684	-4.4	--	--
b13	.SAVTALWGKVNVD.e	1341.716	-1.5	--	--
b14	.SAVTALWGKVNDE.v	1470.759	-1.4	--	--
b16	.SAVTALWGKVNDEVG.g	1626.856	4.6	--	--
b2	.SA.v	159.076	-0.9	--	--
b4	.SAVT.a	359.193	0.4	--	--
b6	.SAVTAL.w	543.312	-3.3	--	--
b7	.SAVTALW.g	729.393	-0.2	--	--
b8	.SAVTALWG.k	786.411	-4.1	--	--
b9	.SAVTALWGK.v	914.509	2.2	--	--
y11	w.GKVNDEVGGK.	1101.589	-1.2	--	--
y12	l.WGKVNDEVGGK.	1287.667	-1.4	--	--
y13	a.LWGKVNDEVGGK.	1400.754	0.3	--	--
y15	v.TALWGKVNDEVGGK.	1572.836	-1.2	--	--
y6	v.DEVGGK.	604.291	-4.1	--	--
y7	n.VDEVGGK.	703.366	4.9	--	--
y8	v.NDEVGGK.	817.405	0.3	--	--

y9	k.VNVDEVGGK.	916.471	-2.1	--	--
Sample 848	theoretical m/z 1377.7164	ID HBD Punjab/Los Angeles (121-133)			
Ion fragm.	sequence	m/z (ttfleX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)
MH+	QFTPPVQAAYQK	1377.712	-2.8		
b2	.QF.t	276.134	-1.4	NA	NA
b3	.QFT.p	377.181	-2.8	NA	NA
b4	.QFTP.p	474.234	-1.8	NA	NA
b5	.QFTPP.v	571.289	2.7	NA	NA
b6	.QFTPPV.q	670.355	-1.9	NA	NA
b7	.QFTPPVQ.a	798.413	-2.4	NA	NA
b8	.QFTPPVQA.a	869.448	-4.1	NA	NA
b9	.QFTPPVQAA.y	940.491	-3.4	NA	NA
b10	.QFTPPVQAAY.q	1103.550	-1.7	NA	NA
b11	.QFTPPVQAAYQ.k	1231.614	2.6	NA	NA
y2	y.QK.	275.171	-2.5	NA	NA
y3	a.YQK.	438.235	-0.5	NA	NA
y4	a.AYQK.	509.270	-2.7	NA	NA
y6	v.QAAYQK.	708.368	0.2	NA	NA
y8	p.PVQAAYQK.	904.491	2.4	NA	NA
y9	t.PPVQAAYQK.	1001.540	-1.1	NA	NA
y10	f.TPPVQAAYQK.	1102.588	-1.1	NA	NA
y11	q.FTPPVQAAYQK.	1249.653	-3.3	NA	NA
a7	.QFTPPVQ.a	770.420	0.1	NA	NA
Sample 850	theoretical m/z 2286.1726	ID HBJ Baltimore (10-31)			
Ion fragm.	sequence	m/z (ttfleX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)
MH+	SAVTALWDKVVNVDEVGGEALGR	2286.129	-1.7	2286.159	-5.8
b5	.SAVTA.l	430.227	-5	--	--
b6	.SAVTAL.w	543.311	-4.2	--	--
b10	.SAVTALWDKV.n	1071.583	-0.7	--	--
b11	.SAVTALWDKVN.v	1185.623	-2.8	--	--
b12	.SAVTALWDKVVN.d	1284.693	-1.6	--	--
b13	.SAVTALWDKVVND.e	1399.722	-1.9	--	--
b15	.SAVTALWDKVVNDEV.g	1627.831	-0.8	--	--
b18	.SAVTALWDKVVNVDEVGGE.a	1870.923	2.4	--	--
b21	.SAVTALWDKVVNVDEVGGEALG.r	2112.056	-2.4	--	--
y4	e.ALGR.	416.262	-4.4	--	--
y6	g.GEALGR.	602.324	-2.2	602.324	-3.0
y7	v.GGEALGR.	659.345	-2.6	659.338	13.1
y8	e.VGGEALGR.	758.414	-1.9	758.420	6.3
y9	d.EVGGEALGR.	887.455	-3	887.456	-2.6
y10	v.DEVGGEALGR.	1002.484	-0.7	--	--

y11	n.VDEVGGGEALGR.	1101.549	-4.3	--	--
y12	v.NVDEVGGGEALGR.	1215.595	-1.3	--	--
y13	k.VNVDEVGGGEALGR.	1314.661	-2.8	--	--
y14	d.KNVDEVGGGEALGR.	1442.756	-2.7	1442.760	6.3
y15	w.DKVNDEVGGGEALGR.	1557.789	1.5	--	--
y16	l.WDKVNDEVGGGEALGR.	1743.866	0.1	--	--
y18	t.ALWDKVNDEVGGGEALGR.	1927.986	-0.8	--	--
Sample 850	theoretical m/z 1313.6812	ID HBD Iran (19-31)			
Ion fragm.	sequence	m/z (ttfleX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)
MH+	.VNVDQVGGEALGR.	1313.677	-2.8	NA	NA
b2	.VN.v	214.118	-2.5	NA	NA
b6	.VNVDQV.g	655.339	-2.9	NA	NA
b8	.VNVDQVGGE.e	769.383	-1.2	NA	NA
y3	a.LGR.	345.223	-3.6	NA	NA
y4	e.ALGR.	416.260	-4.8	NA	NA
y6	g.GEALGR.	602.324	-2.2	NA	NA
y7	v.GGEALGR.	659.345	-2.8	NA	NA
y8	q.VGGEALGR.	758.414	-1.9	NA	NA
y9	d.QVGGEALGR.	886.471	-3.4	NA	NA
y10	v.DQVGGEALGR.	1001.503	2.3	NA	NA
y11	n.VDQVGGEALGR.	1100.568	-3	NA	NA
a5	.VNVDQ.v	528.278	-0.9	NA	NA
a6	.VNVDQV.g	627.345	-1.4	NA	NA
a8	.VNVDQVGGE.e	741.389	0.2	NA	NA
Sample 851	theoretical m/z 2227.1831	ID HBD Iran (10-31)			
Ion fragm.	sequence	m/z (ttfleX)	accuracy (ppm)	m/z (Synapt)	accuracy (ppm)
MH+	SAVTALWGKNVDQVGGEALGR	2227.178	-2.2	2227.178	-2.2
b5	.SAVTA.l	430.23	-2.3	--	--
b6	.SAVTAL.w	543.314	-2.6	543.309	-8.1
b7	.SAVTALW.g	729.393	-2.2	--	--
b8	.SAVTALWG.k	786.414	-2.2	--	--
b9	.SAVTALWGK.v	914.509	-2.5	--	--
b10	.SAVTALWGKV.n	1013.578	-2.4	--	--
b11	.SAVTALWGKVN.v	1127.621	-2.3	1127.604	-14.8
b12	.SAVTALWGKVVN.d	1226.689	-3.5		
b13	.SAVTALWGKVNVD.q	1341.716	-2	1341.727	7.8
b15	.SAVTALWGKVNVDQV.g	1568.843	-1.9	--	--
b18	.SAVTALWGKVNVDQVGGE.a	1811.929	-1.9	--	--
b21	.SAVTALWGKVNVDQVGGEALG.r	2053.071	-2.1	--	--
y4	e.ALGR.	416.262	-4.3	416.261	-1.4
y5	g.EALGR.	545.304	-0.4	--	--
y6	g.GEALGR.	602.326	-2.2	--	--

y7	v.GGEALGR.	659.347	-2.8	--	--
y8	q.VGGEALGR.	758.416	-2.1	--	--
y10	v.DQVGGEALGR.	1001.501	-1.4	--	--
y11	n.VDQVGGEALGR.	1100.569	-1.8	--	--
y12	v.NVDQVGGEALGR.	1214.612	-2.7	--	--
y13	k.VNVDQVGGEALGR.	1313.681	-2.1	--	--
y14	g.KVNVDQVGGEALGR.	1441.776	-2.3	--	--
y15	w.GKVNVDQVGGEALGR.	1498.797	-1.9	--	--
y16	l.WGKVNVDQVGGEALGR.	1684.877	1.6	--	--
y17	a.LWGKVNVDQVGGEALGR.	1797.961	0.2	--	--
a7	.SAVTALW.g	701.398	-0.1	--	--
a8	.SAVTALWG.k	--	--	758.420	-1.0
a10	.SAVTALWGKV.n	985.583	-0.9	--	--
a12	.SAVTALWGKVV.d	1198.694	-2.2	--	--
c21	.SAVTALWGKVNVDQVGGEALG.r	2070.098	-1.3	--	--

Table S3. ttleX and Synapt MALDI MS/MS assignments to Hb variants detected in patients' blood samples 843-844, 846-848, 850-851. The symbol "--" means no fragment ion detected in the MS/MS spectrum. No variants were detected in patient 849 on either of the two instruments and hence no MS/MS data were collected. No variants were detected for patient 848 on the Synapt and hence the *m/z* Synapt column shows "NA". "NA" has been used also when MS/MS data were not collected due the parent ion not being detected in MS mode.

HB variant and proteotypic peptide	proteotypic peptide sequence	Swiss Prot peptide search. Match results	Blast (100% homology results)
HBC (2-18)	VHLTPKESAVTALWVGK	human	none
HBC (2-9)	VHLTPKEK	none	no hits
HBC (2-7)	VHLTPK	Fungi/Metazoa/human (Thyroid adenoma-associated protein)/bacteria	no hits
HBS (2-9)	VHLTPVEK		none
HBS(2-18)	VHLTPVESAVTALWVGK	Human	none
HBE (19-41)	VNVDEVGGKALGRLLVV YPW TQR	none	none
HBE (10-31)	SAVTALWGKVNVDVGGKAL GR	Human	none
HBE (10-27)	SAVTALWGKVNVDVGGK	Human	100% human and primates - β Haemoglobin
HBE (19-31)	VNVDEVGGKALGR	Human	none
HBE (19-27)	VNVDEVGGK	Human	no hits
HbD-Punjab/Los Angeles (121-145)	QFTPPVQAAYQKVAG VANA LAHK	Human	none
HbD-Punjab/Los Angeles (121-147)	QFTPPVQAAYQKVAG VANA LAHKYH	none	none
HbD-Punjab/Los Angeles (121-133)	QFTPPVQAAYQK	none	none
HbD-Iran (19-41)	VNVDQVGGEALGRLLV VYPW TQR	none	none
HbD-Iran (10-31)	SAVTALWGKVNVDQVG GEAL GR	none	none
HbD-Iran (19-31)	VNVDQVGGEALGR	none	none
HbJ-Baltimore (10-31)	SAVTALWDKVNVDVGEAL GR	none	100% homology Gorilla gorilla - β Haemoglobin
HbJ-Baltimore (1-18)	MVHLTPEEKSAVTALW DK	none	none
HbJ-Baltimore (10-18)	SAVTALWDK	none	no hits

Table S4. Specificity of Hb var proteotypic peptides investigated over the entire complement of proteins and species included in the SwissProt database version 2020_2021.