

UK NEQAS for H&I Scheme 5A - HFE Typing

SAMPLES 5A06-5A10/2016

DISPATCHED ON 14TH JUNE 2016

METHODOLOGY

Lab. No.	Typing methods used	Primer / oligo source	Detection method used	Reference to primer / oligo sequences	Comments on HFE typing method	Other HFE mutations or associated polymorphisms
1	PCR-SSP	Alta Bioscience	Gel			No
4	PCR by light cycler and melting curve analysis	Roche diagnostic	Melting curve analysis	Mangasser-Stephan, K (1999), Rapid genotyping of gene mutations with fluorescent hybridisation probes. Clinical chemistry 45, No 12	Both mutations are assayed in the same capillary tube	No
5	Lightcycler 480 melt curve genotyping	Roche-Tib MolBiol lightmixer Kit	Fluorescence			No
13	PCR-RFLP	Sigma	Standard gel			No
14	PCR-RFLP	Life Technologies	3% agarose gel	Feder et al 1996		No
15	PCR-SSP	In-house	Standard gel			No
17	PCR-SSP	In-house	Standard gel	Mulligan et al, GUT (1998), 42 (4), 566-569		No
19	PCR-SSP	In-house	Standard gel			No
22	RT-PCR Fret probe melting curve analysis	Primers - MWG eurofins Probes - Sigma Aldrich	Fluorescence	Meadows et al- RT-PCR - Springer 2001		No
33	RT-PCR - Melting curve analysis	Tib MolBiol lightmix	RT-PCR Fluorescence Roche lightcycler 480 II			No
34	PCR-SSP		Standard gel	Gutteridge et al, 1997, Vox Sanguinis		No
35	PCR-SSP, Taqman	Life Technologies	Standard gel, Fluorescence	Mulligan et al, 1998, GUT:42, 566-569 Kok et al, 2002, Human Mutation:19, 554-559 Cukjati et al, 2007, BMC Medical Genetics: 8, 69-78		No
36						
37	PCR-RFLP	Sigma	Agarose gel, gel red fluorescence			No
39	PCR-SSP	In-house	Standard gel			No
42	PCR-SSP	Integrated DNA Technologies	Agarose gel/Safeview Stain			No
43	PCR - Melt curve analysis	Tib Biol/Roche	Fluorescence			No
48	PCR-SSP	Eurogentec	Standard gel	Gurtridge, Vox SANG 75, 1998		No
49	Allelic discrimination	Taqman	RT-fast PCR 7500			No
50	ARMS-PCR	MWG eurofins	Agarose gel + EB staining	Baty et al. J Clin Pathol 1998:51:73-74		No
52	ARMS-PCR	Thermo	Standard gel	Baty et al. J Clin Pathol 1998:51:73-74		No
53	Sanger sequencing	M13 tagged primers	Fluorescent sanger sequencing on ABI3730			No
55	PCR-SSP	Hain	Melt curve analysis - Fluorescence			No
56	Amplicon-based next generation sequencing	In-house	Illumina MiSeq			No
59	Restriction enzyme PCR	Eurofins	Standard gel			No
62	PCR-SSP	Sigma Aldrich	Standard agarose gel			No
63	Taqman AD	Life Technologies	RT-PCR Fluorescence	RT-PCR custom primers/probes		IVS3 + 1G/T
64	Agena Sequenom	Metabion	Tandem-MS based assay			No
65	PCR and allelic discrimination by Taqman probe/5' nuclease	Life Technologies/Applied Biosystems	Fluorescence	NCBI dbSNP, rs1800562 and rs1799945		No
70	RT-PCR - Lightcycler 480	Tib MolBiol	Fluorescence			No
74	PCR and Sequencing	Eurogentec				No
78	Allelic discrimination	ABI	qPCR			No
79	Taqman	Sigma and Exiqon primers, IDT probes	Fluorescence			Full gene sequencing using NGS for HFE and other related iron genes
80	PCR lightcycler, Melt curve analysis	Genes 4U, Ratiogen AG	Fluorescence melt curve analysis	Genes 4U C282Y + H63D/S65C toolsets		No
81	Lightcycler FRET melt analysis	Sigma Aldrich/Tib MolBiol	Fluorescence	Mangasser-Stephen et al (1998) Clinical Chemistry 45(10), 1875-75. With modified mutation probe for H63D and modified primers for C282Y		No
84	PCR-Fluorogenic target-specific hybridisation and melting curve analysis	Tib MolBiol	Fluorescence			No
85	RT-PCR	In-house	Fluorescence			No
86	RT-PCR	Life Technologies	Fluorescence			No
88	SNP-PCR (RealTime allelic discrimination)	Applied Biosystems	RT-PCR			No
89	Lightcycler PCS melting curve	Tib MolBiol	Melting curve			No
91	PCR enzyme digest	Eurogentec	Standard gel	Journal of Medical Genetics, April 2007, Vol 34, No 4, pp 275-278		No
92	PCR lightcycler - Roche melting curve analysis	Tib MolBiol, Ratiogen	Fluorescence	Mangasser assay, Genes 4U		S65
94	Melt curve analysis	Metabion	Lightscanner	Zhou et al (2004) Clinical Chemistry 50:1328-1335		No
95	RT-PCR	Invitrogen/Tib	Melting curve analysis			No
96	RT-Allelic specific PCR	Euroclone diagnostic	Fluorescence			No
97	Hybridisation and simple probes used in Multiplex PCR	Tib MolBiol lightmix HFE kit	Fluorescence			No
99	RT-PCR	Euroclone Haemochromatosis H63D and C282Y genotyping kits	RT-PCR fluorescence detection			No
108	RT-PCR	Applied Biosystems	Fluorescence			No
138	RT-PCR HFE mutation detected by fluorescent hibridazion probe melting curves	Tib MolBiol (Roche)	Fluorescence	Tib MolBiol synthese (Roche) detection of HFE mutations		No
150	Sequencing	In-house				Exon 2 and Exon 4
154	PCR-SSO	Commercial	Hybridation on mambrane			V53M, V59M, H63H, Q127H, P160delC, E168Q, E168X, W169X, Q283P, E60X, M172K, Y250X, AVAQ594-597del, N144H, V162del Codon 168 (E and/or X)
269	PCR-SSOP	Euroimmun	Fluorescence			No
270	PCR-RFLP	In-house	Acrylamide gel and BET coloration			No
282	RT-PCR	Sigma Aldrich Prologo	Fluorescence			No
288	HRM technology followed by sequencing (Sanger)	Primers were from Eurofins	Fluorescence for HRM		Sequencing is done only when HRM presents an altered profile suggesting homozygous or heterozygous mutation	No
314	Taqman RT-PCR	Applied biosystems	Fluorescence			No
327	PCR and hybridation reverse	Viennalab.ref4-220 haemochromatosis stripassay A	Hybridation sur bandelette	Viennalab.ref4-220 haemochromatosis stripassay A		No
331	RT-PCR (allelic discrimination)	Probes Apletra (Applied Biosystems) and primers Sigma Aldrich	Fluorescence	Jacques B. et al. Human mutation 19:554-559, 2002		No

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RESULTS

Consensus:			HH	CC	SS	DD	CC	SS	HD	CC	SS	HH	YY	SS	HH	YY	SS	Comments	
Lab. No.	Assessment			Codon 63	Codon 282	Codon 65	Codon 63	Codon 282	Codon 65	Codon 63	Codon 282	Codon 65	Codon 63	Codon 282	Codon 65	Codon 63	Codon 282	Codon 65	
	63	282	65	5A06/2016	5A06/2016	5A06/2016	5A07/2016	5A07/2016	5A07/2016	5A08/2016	5A08/2016	5A08/2016	5A09/2016	5A09/2016	5A09/2016	5A10/2016	5A10/2016	5A10/2016	
1	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
4	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
5	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
13	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
14	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
15	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
17	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
19	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
22	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
33	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
34	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
35	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
36	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
37	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
39	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
42	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
43	YES	YES	YES	NT	NT	NT	DD	CC	HD	CC	NT	HH	YY	NT	HH	YY	NT	No samples received	
48	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
49	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
50	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
52	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
53	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
55	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
56	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
59	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
62	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
63	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
64	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
65	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
70	YES	YES		HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
74	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
78	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
79	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
80	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
81	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
84	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
85	YES	YES		HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
86	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
88	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
89	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
91	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
92	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
94	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
95	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
96	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
97	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
99	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
108	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
138	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
150	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
154	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
269	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
270	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
282	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
288	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
314	YES	YES		HH	CC		DD	CC	HD	CC		HH	YY		HH	YY			
327	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		
331	YES	YES	YES	HH	CC	SS	DD	CC	HD	CC	SS	HH	YY	SS	HH	YY	SS		