

UK NEQAS FOR H&I SCHEME 5A - HFE TYPING

HFE METHODOLOGY RESULTS OF SAMPLE 5A01-5A05/2014

DESPATCHED ON 28th JANUARY 2014

Consensus HD CY SS HH CC SS HH YY SS HD CC SS HH CC SS

Lab. No.	Codon 63 5A01/14	Codon 282 5A01/14	Codon 65 5A01/14	Codon 63 5A02/14	Codon 282 5A02/14	Codon 65 5A02/14	Codon 63 5A03/14	Codon 282 5A03/14	Codon 65 5A03/14	Codon 63 5A04/14	Codon 282 5A04/14	Codon 65 5A04/14	Codon 63 5A05/14	Codon 282 5A05/14	Codon 65 5A05/14	Date Received	Date Tested	Comments
1	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	13-Feb	
2	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	15-Feb	
4	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	06-Feb	
5	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	05-Feb	
13	HD	CC	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	04-Feb	
14	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	30-Jan	
15	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan		
17	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	29-Jan	
19	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	30-Jan	
20	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	31-Jan	
22	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	30-Jan	
33	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	31-Jan	06-Feb	
34	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	03-Feb	
35	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	30-Jan	24-Feb	
36	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC			13-Feb	
37	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC				
39	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	30-Jan	
42	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	31-Jan	
43	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS		27-Feb	
44	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		12-Feb	21-Feb	
48	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		31-Jan	03-Feb	
49	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	06-Feb	
50	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	03-Feb	
52	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	10-Feb	
53	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	18-Feb	
56	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	10-Feb	
59	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	03-Feb	
61	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	10-Feb	
62	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	03-Feb	11-Feb	
63	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	31-Jan	
64	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	30-Jan	17-Feb	
65	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		29-Jan	03-Feb	
74	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	06-Feb	
78	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	30-Jan	31-Jan	
79	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	30-Jan	05-Feb	
80	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		10-Feb	18-Feb	
81	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	03-Feb	12-Feb	
84	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	06-Feb	
85	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	01-Feb	
86	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	05-Feb	
																30-Jan	10-Mar	
87		CY			CC			YY			CC			CC				There was poor amplification for the H63S/S65C products. These will be repeated in the next run. It is not financially permitted to repeat these in a run on their own. Results will be checked once repeated.
88	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	04-Feb	
89	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC				
91	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	17-Feb	
92	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	03-Feb	07-Feb	
94	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	30-Jan	04-Feb	
95	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS		25-Feb	
96	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		31-Jan	04-Feb	
97	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	31-Jan	18-Feb	
99	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		03-Feb	05-Feb	
103	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	07-Feb	
108	HD	CY		HH	CC		HH	YY		HD	CC		HH	CC		30-Jan	05-Feb	
138	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	31-Jan	07-Feb	
140	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT			
150	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	05-Feb	07-Feb	
154	HD	CY	SS	HH	CC	SS	HH	YY	SS	HD	CC	SS	HH	CC	SS	29-Jan	30-Jan	
268		CY		HH	CC		HH	YY		HD	CC		HH	CC		03-Feb	08-Feb	

UK NEQAS FOR H&I SCHEME 5 - HFE TYPING

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Lab. No.	Typing methods used	Primer / oligo source	Detection method used	Reference to primer / oligo sequences
1	Melting curve analysis on the lightcycler	TibMolBiol	Fluorescence	
2	RT-PCR	Euroclone	Fluorescence	
4	PCR-SSP + Melting curve analysis	Roche	Fluorescence probe method	
5	Lightcycler 480 melting genotyping	Roche - TibMolBiol lightmix kit	Fluorescence	
13	PCR-RFLP	Sigma	Agarose gel	
14	PCR-RFLP	Life Technologies	3% Agarose gel	As previous
15	PCR-SSP	In-house	Gel	
17	PCR-SSP	In-house	Agarose gel	Mulligan et al GUT (1998); 42(4):566-569
19	PCR-SSP	In-house	Standard gel	Smillie J. Mol Path (1998)
20				
22	PCR fret probe melting curve analysis	Primers-MWG: Probes - Sigma Aldridge	Fluorescence	Meadows et al - Real time PCR - Springer 2001
30				
33	RT-PCR melt curve analysis	TibMolBiol lightmix kit	RT-PCR fluorescence Roche light cycler 480	
34	PCR-SSP		Standard gel	Gutteridge et al
	PCR-SSP, Taqman	Life Technologies	Fluorescence, Standard gel	Mulligan et al GUT (1998); 42:566-569 Kok et al 2002; Human Mutation; 19:554-559 Cukjato et al 2007; BMC Medical Genetics; 8:69-78
35				
36	PCR	Lightmix Roche TibMolBiol	Fluorescence	
37	PCR-RFLP	Sigma	Agarose gel, Gel red fluorescence	
39	PCR-SSP	In-house	Standard gel	
42	PCR-SSP	Integrated DNA Technologies	Agarose gel, Safeview stain	
43	PCR melt curve genotyping	TibMolBiol Roche HFE duplex kit	Fluorescence	
44	RT-PCR	Euroclone	Fluorescence	
48	PCR-SSP	Eurogentec	Standard gel	Gurtridge, Vox Sang, 75, 1998
49	RT-PCR Allelic Discrimination	AB Life Technologies	Fluorescence	
50	ARMS-PCR	MWG	Agarose gel + EB	Baty et al 1998 J. Clin Pathol; 51:73-74
52	ARMS-PCR	Thermo	Standard gel	Baty et al 1998 J. Clin Pathol; 51:73-74
53				
	PCR-Restriction Deigest	Thermo Electron	2.5% fluorescent gel	Feder 1996 (Nat Genet 13:399) Jeffery 1999 (Nat Genet 22:325%)
56				
59	RT-PCR	Eurofins	Standard gel	
61	Allele-Specific PCR	Integrated DNA Technologies	Capillary Electrophoresis	Gomez-Llorente et al, Eur. J. Haematol (2004); 72:121-129
62	PCR-SSP	Eurogentec	Standard agarose gel	
	C282Y + H63D real time PCR S65C	ABI	Fluorescence and enzyme digestion + gel electrophoresis	ABI probes; C282Y C-1085595-10 H53D-1085600-10 H63D F + H63D R, Alison May
63	restriction enzyme digest			
64	PCR-SSCP technique		SDS-Page and Silver staining	
	PCR and allelic discrimination by	Life Technologies / applied Biosystems	Fluorescence	NCBI db SNP rs1800562 and rs1799945
65	TaqMan 5' nuclease			
74	Bi-directional Sequencing	Eurogentec	Fluorescence	
78	PCR-SSP	In-house	Standard agarose gel	
79	TaqMan	Sigma + Exiqon primers, IDT probes	Fluorescence	
80	PCR lightcycler, Melt curve analysis	Genes-4U	Fluorescence melt curve analysis	Genes 4U C282Y + H63D/S65C toolsets
81	PCR (fret probes)	Modified from prior publication	Fluorescence	
	PCR-Fluologenic target-specific	TibMolBiol	Fluorescence	
84	hybridisation + melting curve analysis			
85	RT-PCR	Life Technologies	Fluorescence	
86	RT-PCR	Life Technologies	Fluorescence	Assay by design
87	Lightcycler - Fluorescent PCR	HFE Lightmix	Fluorescence	
	Fluorescence allele-specific	Applied Biosystems	Fluorescence	
88	discrimination			
89	Lightcycler PCR melting curves	TibMolBiol	Melting curves	
91	PCR-Enzyme digests	Eurogentec	Standard gel	Journal of medical genetics, April 2007 vol 34, no 4 pp275-278
	PCR lightcycler Roche melting curve	C282-TibMolBiol, Genes-4U kit for HFE 63/65	Fluorescence	C282-Mangasser assay H63D+S65C toolset for lightcycler gener-4u
92	analysis			
	Melt curve analysis (Luna probes on	Metabion	Fluorescence	Zhou et al (2004) Clinical Chemistry 50:1328-1335
94	Lightscanner)			
95	RT-PCR	Invitrogen/TIB	Melting curve analysis	
96	RT-PCR	Euroclone	Fluorescence	
97	RT-PCR	TibMolBiol	Fluorescence	
	RT-PCR	Euroclone Haemochromatosis H63D & C282Y genotyping kits	RT-PCR fluorescence detection	
99				
	PCR TaqMan (ABI Prism 7500) with MGB probes (C282Y and H63D)	MWG for primers and Applera for MGB probes Taqman	Fluorescence	Rapid genotyping of single nucleotide polymorphisms using novel minor groove binding DNA oligonucleotides (MGB probes) - Jacques B de Kok et al, Human Mutation 19:554-559(2002)
103				
108	RT-PCR	Applied Biosystems	Fluorescence	
	PCR-SSP, SSO, SBT	RT PCR HFE mutations detected by fluorescent hybridisation probe melting curves	LC red 640, LC red 705, fluorescen	TibMolBiol synthese (Roche), detection C282Y, H63D, S65C
138				
140				
150	Sequencing	In-house		
154	PCR-SSO	Constructor	Hybridation on mambrane	
	Amplicon genotyping by high resolution melt analysis. Positive samples are double checked by TaqMan SN6 genotyping (minor groove binder technology)	In-house	Fluorescence	
268				
269	PCR-SSP & Hybridisation	HFE gene	Fluorescence	
270	PCR-RFLP	Eurogentec	Standard gel (acrylamid) / EB	
282	RT-PCR	Sigma aldrich proligo	Fluorescence	

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Lab. No.	Comments on HFE typing method	Typing for other HFE mutations or associated polymorphisms
1		No
2		No
4		No
5		No
13		No
14		No
15		No
17		No
19		No
20		
22		
30		
33		No
34		No
35		No
36		No
37		No
39		No
42		No
43		No
44		No
48		No
49		No
50		No
52		No
53		
56	C282Y F primer changed from above paper due to SNP. Restriction enzymes: SnaBI-(C282Y mutation) NDEII-(H63D mutation).	No
59		No
61	All referrals are tested for the p.C282Y variant. Only those identified as heterozygous or those with a stated family history are then activated for testing for the p.H63D variant	No
62		Yes - IVS3 + 1G/T
63		Yes - S65C
64		Yes - S65C
65		No
74		No
78		No
79		Yes - Full gene sequencing
80		No
81		No
84		No
85		No
86		No
87		
88		No
89		No
91		No
92		Yes - S65 using Genes-4U kit
94		No
95		Yes - S65C
96		No
97		No
99		No
103		Yes - S65C - PCR-RFLP, Mura et al, 1999 Blood 93:2502-2505
108		No
138		No
140		
150		Yes - All mutations in exon 2 and 4
154		Yes - HFE - V53M, V59M, H63D, H63H, S65C, Q127H, P160delC, E168Q, E168X, W169X, C282Y, Q283P TFR2: E60X, M172K, Y250X, AVAQ594-597del FPN1: N144H, V162del
268		Yes - H63D (187 C>G), S65C (193 A>T), C282Y (845 G>A), E168X (502 G>T)
269		No
270		No
282		No