

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

HFE METHODOLOGY RESULTS OF SAMPLE 5A06-5A10/2014

DESPATCHED ON 10TH JUNE 2014

Consensus	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	Date Received	Date Tested	Comments
Lab. No.	Codon 63 5A06/14	Codon 282 5A06/14	Codon 65 5A06/14	Codon 63 5A07/14	Codon 282 5A07/14	Codon 65 5A07/14	Codon 63 5A08/14	Codon 282 5A08/14	Codon 65 5A08/14	Codon 63 5A09/14	Codon 282 5A09/14	Codon 65 5A09/14	Codon 63 5A10/14	Codon 282 5A10/14	Codon 65 5A10/14			
1	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		13-Jun	19-Jun	
2	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		12-Jun	19-Jun	
4	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	24-Jun	
5	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	13-Jun	
13	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	18-Jun	
14	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	24-Jun	
15	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	16-Jun	
17	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	18-Jun	
19	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	11-Jun	
20	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	17-Jun	
22	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	16-Jun	
33	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	20-Jun	03-Jul	
34	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun		
35	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	12-Jun	27-Jun	
36	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY			01-Jul	
37	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	16-Jun	
39	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		12-Jun	19-Jun	
42	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		12-Jun	17-Jun	
43	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	19-Jun	
48	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		12-Jun	17-Jun	
49	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY			12-Jun	
50	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	17-Jun	
52	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		12-Jun	24-Jun	
53	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	27-Jun	
56	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	17-Jun	
59	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		13-Jun	18-Jun	
61	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		19-Jun	23-Jun	
62	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	17-Jun	
63	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	12-Jun	23-Jun	
64	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	24-Jun	
65	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	25-Jun	
74	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	08-Jul	
78	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	18-Jun	
79	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	18-Jun	
80	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		24-Jun	30-Jun	
81	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	13-Jun	
84	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	12-Jun	02-Jul	
85	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	12-Jun	16-Jun	
86	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		16-Jun	20-Jun	
87	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS		25-Jul	
88	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		13-Jun	24-Jun	
89	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		15-Jun	25-Jun	
91	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		12-Jun	18-Jun	
92	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	16-Jun	02-Jul	
94	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun	20-Jun	
95	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS		03-Jul	
96	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		10-Jun	11-Jun	
97	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	16-Jun	19-Jun	
99	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		11-Jun	26-Jun	
103	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	11-Jun		
108	HH	CC		HD	CY		HH	CC		HH	CC		HH	CY		13-Jun	24-Jun	
138	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	17-Jun		
150	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	18-Jun	21-Jun	
154	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	13-Jun	02-Jul	No other HFE mutations were found for every sample
268		CC			CY			CC			CC			CY		16-Jun	21-Jun	
269	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	12-Jun	17-Jun	E168X results: 5A06 - EE 5A07 - EE 5A08 - EE 5A09 - EE 5A10 - EE
270	HH	CC	SS	HD	CY	SS	HH	CC	SS	HH	CC	SS	HH	CY	SS	12-Jun	16-Jun	

**UK NEQAS FOR HLA SCHEME 5 - HFE TYPING**

HFE METHODOLOGY RESULTS OF SAMPLE 5A06-5A10/2014

DESPATCHED ON 10TH JUNE 2014

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						No
2	RT-PCR	Euroclone	Fluorescence			No
4	PCR-SSP + melting curve analysis	Molbiol	Fluorescence probe method	Mangasser-Stephan et al (1999) Rapid genotyping of haemochromatosis gene mutation with fluorescent hybridisation probes. Clinical chemistry 45:10 (1875-1878)		No
5	Lightcycler 480 meltcurve genotyping	Roch-Tib Molbiol lightmix kit	Fluorescence			No
13	PCR-RFLP	Sigma	Agarose gel			No
14	PCR-RFLP	LifeTech/Fisher	5% Agarose gel	Feder et al. 1996		No
15	PCR-SSP + melting curve analysis	In-house	Standard gel			No
17	PCR-SSP	In-house	Agarose gel	Mulligan et al. GUT (1996), 42 (4) 566-569		No
19	PCR-SSP	In-house	Standard gel	Smille J. Mol Path (1998)		No
20	PCR-SSP	In-house	Gel			No
22	PCR het probe melting curve analysis	Primers - MWG	Fluorescence	Meadows et al RT-PCR - Springer 2001		No
33	RT-PCR - melting curve analysis	Tib Molbiol Lightmix kit	RT-PCR fluorescence Roche lightcycler 480 II			No
34	PCR-SSP					No
35	PCR-SSP, Taqman	Life Technologies	Fluorescence, Standard gel	Mulligan et al. 1998; GUT, 42:566-569 Kok et al 2002; Human Mutation; 19:554-559 Cukpati et al 2007; BMC Medical Genetics; 8:69-78		No
36	PCR	Lightmix Roche Tib Molbiol	Fluorescence			No
37	PCR-RFLP	Sigma	Gel red fluorescence, Agarose gel			No
38	PCR-SSP	In-house	Standard gel			No
42	PCR-SSP	Integrated DNA Technologies	Safeview Stain/Agarose gel			No
43	PCR-Melt curve genotyping	Tib Biol/Roche HFE duplex kit	Fluorescence			No
48	PCR-SSP	Eurogentec	Standard gel			No
49	PCR-Allelic discrimination	AB life technologies	RT-PCR	Gutridge, Vox sang 75 1998 Fast-7500 RT-Fluorescence PCR allelic discrimination		No
50	Arms-PCR	MWG	EB staining + Agarose gel	Baty et al 1998 J.Clin Pathol 51; 73-74		No
52	Arms-PCR	Thermo	Agarose gel	Baty et al 1998 J.Clin Pathol 51; 73-74		No
53	PCR + Sanger Sequencing	Fisher	Bidirectional sequencing + fluorescence detection analysis with mutation surveyor	H63D primers as described in Nat. Genet (13:4) Aug 1996 399-408 C282Y in-house design to avoid primer binding site SNP		No
56	PCR-based next generation sequencing	In-house	Illumina MiSeq next generation sequencing	N/A - However the primers have been checked for polymorphisms using SNPcheck3 ( <a href="https://secure.ngf.org.uk/SNPcheck">https://secure.ngf.org.uk/SNPcheck</a> )	HGS data is analysed through an in-house bioinformatic pipeline to produce the results in a user-friendly format	No
59	PCR-RE	Eurofins	Standard gel			No
61	Allele-specific PRC	Integrated DNA Technologies	Capillary Electrophoresis	Gomez-Llorente et al. EUR. J. Haematol (2004) 72:121-129		No
63	PCR-SSP	Eurogentec	Standard agarose gel			No
63	7500 fast RT-PCR S65C restriction enzyme digestion	ABI	Fluorescence and enzyme digestion	ABI probes customised C282Y C-1080595-10 H63D-C-188600-10 H63D F + H63DR, Alison May	Routine method 7500 fast RT-PCR for C282Y and H63D, S65C by restriction enzyme digest when required	I/S3 + 1GT S65C HFE p.Gln269Pro
64	PCR-SSP					S65C
65	PCR and allelic discrimination by TaqMan 5' nuclease	Life Technologies/Applied biosystems	Fluorescence	NCBI db SNP rs1800562 and rs1799445		No
74	PCR-Sequencing	Eurogentec	Fluorescence			No
78	PCR-SSP	Sigma Genosys	Standard agarose gel			No
79	TaqMan	Sigma + Exiqun primers, IDT probes	Fluorescence			Full gene sequencing
80	PCR, lightcycler, melt curve analysis	Genes-4U	Fluorescent melt curve analysis	Genes-4U C282Y + H63D/S65C probes		No
81	Lightcycler melt analysis	Sigma alidich/Tib Molbiol	Fluorescence	Mangasser-Stephan et al (1999) clinical chemistry 45:10 (1875) with modified mutation probe for H63D		No
84	PCR-Fluorogenic target-specific hybridisation with melting curve analysis	Tib Molbiol/Roche	Fluorescence			No
86	RT-PCR	Life Technologies	Fluorescence			No
86	RT-PCR	Life Technologies	Fluorescence	Assay by design		No
87	Lightcycler PCR	Tib Molbiol	Fluorescence			No
88	Fluorescence allele-specific discrimination	Applied Biosystems	Fluorescence			No
88	Lightcycler melting curve genotyping	Tib Molbiol	Fluorescence			No
91	PCR-Enzyme digest	Eurogentec Ltd	Standard gel	Hjournal of Medical Genetics, April 2007, Vol 34, No 4, p.275-278		No
92	PCR lightcycler-Roche melting curve analysis	C282- Tib Molbiol, Genes-4U kit for HFE 63/65	Fluorescence	C282-Mangasser assay H63D + S65C booster for lightcycler - Genes-4U		S65 using Genes-4U kit
94	Melt curve analysis	Metabion	Fluorescence	Zhou et al (2004) Clinical Chemistry 50:1328-1335		No
96	RT-PCR	Invitrogen/TIB	Melting curve analysis	Mangasser-Stephan, K et al (1999) Rapid genotyping of haemochromatosis gene mutations on the light cycler with fluorescent hybridisation probes. Clinical Chemistry 45:1875-1878 Bollhauer, M et al (1999) lightcycler PCR assay for simultaneous detection of the H63D and S65C mutations in the HFE haemochromatosis gene based on opposite melting temperature shifts. Clinical Chemistry 45:2275-2278		S65C
96	RT-PCR	Euroclone	Fluorescence	Not known		No
97	RT-PCR	Tib Molbiol	Fluorescent	Ligh cycler 2.0		No
98	RT-PCR	Euroclone haemochromatosis H63D + C282Y genotyping kit	RT-PCR fluorescence detection			No
103	PCR TaqMan (ABI Prism 7500) with MGB probes (C282Y and H63D)	MWG for primers and Applied 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)		S65C - PCR-RFLP. Mura et al. 1999 Blood 93:2502-2505
108	RT-PCR	Applied Biosystems	Fluorescence			No
138	PCR-SSP, SBT	RT PCR HFE mutations detected by fluorescent hybridisation probe melting curves	LC red 640, LC red 705, fluorescent	TibMol/Biol synthese (Roche), detection C282Y, H63D, S65C		No
150	PCR-Sequencing					V53M, V59M, H63H, Q127H, P160delC, E168Q, E168X, W169X, C269P, E60X, M172X, Y250C, AVA204-57del, N144K, V162del
268	Amplison genotyping by high resolution melt analysis. Positive samples are double checked by TaqMan SNG genotyping	In-house	Fluorescence			No
268	PCR-SSP + hybridisation	HFE gene	Fluorescence			H63D, S65C, C282Y, E168X
270	PCR-RFLP	Eurogentec	Acrylamide - EB			No
282	RT-PCR	Sigma alidich preligo	Fluorescence			No