

UK NEQAS FOR H&I SCHEME 5 - HFE TYPING

HFE METHODOLOGY RESULTS OF SAMPLE 5A01-5A05/2015

DESPATCHED ON 13TH JANUARY 2015

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	Lightcycler and Melting curve analysis	Roche Diagnostic	Melting curve analysis	Mangasser - Stephen, K (1999), Rapid genotyping of haemochromatosis gene mutations with fluorescence hybridisation probes	Both mutations are assayed in the same capillary tube	No
5	Lightcycler 480 meltcurve genotyping	Roche - TibMolBiol lightmix	Fluorescence			No
13	PCR-RFLP	Sigma	Standard gel			No
14	PCR-RFLP	Lifetech	3% agarose gel			No
15	PCR-SSP	In-house	Agarose gel			No
17	PCR-SSP	In-house	Agarose gel	Mulligan et al, GUT (1998), 42 (4), 566-569		No
19	PCR-SSP	In-house	Standard gel	Smillie J. Mol. Path (1998)		No
22	PCR fret probe melting curve analysis	MWG probes - Sigma	Fluorescence	Meadows et al RT-PCR - Springer 2001		No
33	RT-PCR melt curve analysis	TibMolBiol lightmix	Fluorescence			No
34	PCR-SSP		Standard gel			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	PCR	Roche lightmix - TibMolBiol	Fluorescence			No
37	PCR-RFLP	Sigma	Agarose gel, GelRed fluorescence	Feder et al, UK HH Consortium, Jeffreys et al		No
39	PCR-SSP	In-house	Standard gel			No
42	PCR-SSP	Integrated DNA technologies	Agarose gel/Safeview stain			No
43	PCR - melt curve genotyping	TibMolBiol - Roche, HFE duplex	Fluorescence			No
48	PCR-SSP	Eurogentec	Standard gel	Gurtridge we al, Vox SANG, 75 (1998)		No
49	RT-PCR Allelic discrimination	Life Technologies	Fluorescence			No
50	Arms PCR	MWG-Eurosins	Agarose gel + EB	Baty et al (1998) J clin pathol, 51, 73-74		No
52	Arms PCR	Thermo	Standard gel	Baty et al (1998) J clin pathol, 51, 73-74		No
53	Bidirectional sanger sequencing	Invitrogen	Bidirectional sanger sequencing		Sequence data analysed using mutation surveyor software	Other HFE variants of potential clinical significance are reported when identified
55	PCR-SSP	Hain Lifescience GMBH	Fluorescence		Fluorotype' labelled probes characterised by melt curve analysis	No
56	PCR-based next generation sequencing	In-house	Illumina MiSeq NGS		NGS data is analysed through an in-house bioinformatics pipeline to produce the results in a user-friendly format	No
59	PCR-RE	Eurofins	Standard gel			No
61	Allele specific PCR	Integrated DNA technologies	Fluorescence			No
62	PCR-SSP	Sigma-Aldrich	Standard agarose gel			IVS3 + 1G/T
63	7500 fast RT-PCR and PCR restriction enzyme digestion	Life Technologies	Fluorescence, gel electrophoresis	RT-PCR: Customised C282Y, H63D, H63DF+H63DR	Routine method 7500 fast RT-PCR for C282Y and H63D. S65C by restriction enzyme digest when required	HFE sequence analysis if indicated and requested
64	PCR-SSCP		SDS-Page and Silver staining			S65C
65	PCR and allelic discrimination by TaqMan	Life Technologies/Applied	Fluorescence	NCBI db SNP rs1800562 and rs1799945		No
74	PCR/Sequencing	Eurogentec	Fluorescence			No
78	PCR-SSP	Sigma Genosys	Agarose gel			No
79	TaqMan	Sigma + Exiqun primers, IDT	Fluorescence			Full gene sequencing
80	PCR, lightcycler, melt curve analysis	Genes-4U	Fluorescent melt curve analysis	Genes-4U C282Y + H63D/S65C toolset		No
81	Fret analysis	Sigma aldrich/Tib Molbiol	Fluorescence	Mangasser-Stephan et al (1999) clinical chemistry 45:10 (1875) with		No
84	PCR-Fluorogenic target-specific hybridisation with melting curve analysis	Tib Molbiol/Roche	Fluorescence			No
85	RT-PCR	Life Technologies	Taqman			No
86	RT-PCR	Life Technologies	Fluorescence	Assay by design		No
87	Lightcycler PCR	TibMolbiol	Fluorescence	TibMolbiol		No
88	Fluorescence allele-specific discrimination	Applied Biosystems	Fluorescence			No
89	Lightcycler PCR melting curves	TibMolbiol	Melting curve			No
91	PCR-Enzyme digest	Eurogentec	Standard gel	Journal of Medical Genetics, April 2007, Vol 34, No.4, p.275-278		No
92	PCR lightcycler-Roche melting curve analysis	C282- Tib Molbiol, Ratiogen	Fluorescence	C282-Mangasser assay H63D + S65C toolset for lightcycler - Ratiogen		S65 using Genes-4U kit
94	Melt curve analysis	Metabion	Fluorescence			No
95	RT-PCR	Invitrogen/TIB	Melting curve analysis	Zhou et al (2004) Clinical Chemistry 50:1328-1335		No
96	RT-PCR	Euroclone	Fluorescence			No
99	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 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)		S65C - PCR-RFLP, Mura et al, 1999 Blood 93:2502-2505
108	RT-PCR	Applied Biosystems	Fluorescence			No
138	PCR-SSP, PCR-SSO, SBT	RT PCR HFE mutations detected by fluorescent hybridisation probe melting curves	TibMolBiol synthese (Roche)	Detection C282Y, H63D, S65C		No
150	PCR-Sequencing	In-house				No
154	PCR-SSO	Commercial	Hybridation on membrane			V53M, V59M, H63H, Q127H, P160delC, E168Q, E168X, W169X, Q283P, E60X, M172K, Y250X, AVAQ594-597del, N144H, V162del, E168X
269	PCR-SSP + hybridisation	HFE gene	Fluorescence			On request
270	PCR-RFLP	Eurogentec	PCR-RFLP	Stoot et al, Clinical Chemistry (1999), 45:(3), 426-428		No
282	RT-PCR	Sigma aldrich proligo	Fluorescence			No
314	TaqMan RT-PCR assay + sequencing	Life Technologies	Fluorescence			No

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

HFE RESULTS OF SAMPLE 5A01-5A05/2015

DESPATCHED ON 13TH JANUARY 2015

Consensus: HH CC SS HH YY SS HH CC SS HH CC SC HD CC SS

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	Date Received	Date Tested	Comments
	H63D	C282	S65C	5A01/15	5A01/15	5A01/15	5A02/15	5A02/15	5A02/15	5A03/15	5A03/15	5A03/15	5A04/15	5A04/15	5A04/15	5A05/15	5A05/15	5A05/15			
1	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		15-Jan	23-Jan	
2	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		15-Jan	17-Jan	
4	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	26-Jan	
5	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	15-Jan	20-Jan	
13	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	26-Jan	
14	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	20-Jan	
15	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		15-Jan	28-Jan	
17	YES	YES	YES	HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	22-Jan	
19	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		15-Jan	27-Jan	
22	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	19-Jan	19-Jan	
33	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	19-Jan	29-Jan	
34	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	14-Jan	
35	YES	YES		HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	22-Jan	
36	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS		11-Feb	
37	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	26-Jan	
39	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	26-Jan	
42	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC			23-Jan	
43	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS		03-Feb	
48	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		15-Jan	16-Jan	
49	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		20-Jan	04-Feb	
50	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	20-Jan	
52	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	29-Jan	
53	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	15-Jan	27-Jan	
55	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	13-Feb	
56	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	23-Jan	
59	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		15-Jan	27-Jan	
61	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	29-Jan	
62	YES	YES		HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	23-Jan	
63	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	23-Jan	
64	YES	YES		HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	26-Jan	
65	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	21-Jan	
74	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	27-Jan	
78	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	15-Jan	04-Feb	
79	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	19-Jan	
80	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		20-Jan	21-Jan	
81	YES	YES		HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	15-Jan	19-Jan	
84	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	05-Feb	
85	YES	YES		HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS		19-Jan	
86	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		15-Jan	20-Jan	
87	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	26-Jan	04-Feb	
88	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		15-Jan	20-Jan	
89	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC	SC	HD	CC		21-Jan	06-Feb	
91	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	20-Jan	
92	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	15-Jan	21-Jan	
94	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	22-Jan	
95	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	13-Jan	20-Jan	
96	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		14-Jan	28-Jan	
99	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		08-Jan	21-Jan	
103	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	19-Jan	26-Jan	
108	YES	YES		HH	CC		HH	YY		HH	CC		HH	CC		HD	CC		15-Jan	20-Jan	
138	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	19-Jan	
150	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	20-Jan	29-Jan	
154	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	15-Jan	15-Jan	
269	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS			
270	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	14-Jan	19-Jan	
282	YES	YES	YES	HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS			
314	YES	YES		HH	CC	SS	HH	YY	SS	HH	CC	SS	HH	CC	SC	HD	CC	SS	19-Jan	20-Jan	