

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

HFE METHODOLOGY RESULTS OF SAMPLE 5A06-5A10/2015

DISPATCHED ON 02ND JUNE 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	Smilie J. Mol. Path (1998)		No
22	PCR tret 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	Gutridge we al, Vox SANG, 75 (1998)		No
49	RT-PCR Allelic discrimination	Life Technologies	Fluorescence			No
50	Arms PCR	MWG-Euroins	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 form	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			VS3 + 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 5'	Life Technologies/Applied	Fluorescence	NCBI db SNP rs1800562 and rs1799945		No
70	RT-PCR	TibMolBiol	Fluorescence			No
74	PCR/Sequencing	Eurogentec	Fluorescence			No
78	PCR-SSP	Sigma Genosys	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 toolset		No
81	Fret analysis	Sigma aldrich/Tib Molbiol	Fluorescence	Mangasser-Stephan et al (1999) clinical chemistry 45:10		No
84	PCR-Fluoregenic 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 H63D+S65C	Fluorescence	C282-Mangasser assay H63D + S65C toolset for lightcycler - Ratiogen		S65 using Genes-4U kit
94	Melt curve analysis	Metabion	Fluorescence	Zhou et al (2004) Clinical Chemistry 50:1328-1335		No
95	RT-PCR	Invitrogen/TIB	Melting curve analysis			No
96	RT-PCR	Euroclone	Fluorescence			No
99	RT-PCR	Euroclone haemochromatosis H63D + C282Y genotyping kit	RT-PCR fluorescence detection			No
103	NT	NT	NT	NT	NT	NT
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	Hybridation on membrane			No
154	PCR-SSO	Commercial	Hybridation on membrane			VS3M, V59M, H63H, Q127H, P160delC, E168Q, E168X, W169X, Q283P, E60X, M172K, Y250X, AVAG594-597del, 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
327	PCR et hybridation reverse	Viennalab, Ref. 4-220 Haemochromatosis StripAssay A	Hybridation sue bandelette			No
331	RT-PCR	Probes - Applera, Primers - Sigma Aldrich	Fluorescence	Jacques B. et al Human Mutation 19:554-559, 2002		S65C mutation. Mura C. et al Blood 93:2502-2505, 1999

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

Lab. No.	Assessment 63 282 65	Codon 63 5A06/15	Codon 282 5A06/15	Codon 65 5A06/15	Codon 63 5A07/15	Codon 282 5A07/15	Codon 65 5A07/15	Codon 63 5A08/15	Codon 282 5A08/15	Codon 65 5A08/15	Codon 63 5A09/15	Codon 282 5A09/15	Codon 65 5A09/15	Codon 63 5A10/15	Codon 282 5A10/15	Codon 65 5A10/15	Date Received	Date Tested	Comments	
1	YES YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	15-Jun		
2	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		04-Jun	10-Jun		
4	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		02-Jun	12-Jun		
5	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	04-Jun	04-Jun		
13	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	04-Jun		
14	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	08-Jun		
15	YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	15-Jun		
17	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	04-Jun		
19	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	05-Jun		
22	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	05-Jun	08-Jun		
33	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	05-Jun	18-Jun		
34	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	05-Jun		
35	YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	18-Jun		
36	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	12-Jun	24-Jun		
37	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	08-Jun		
39	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		04-Jun	08-Jun		
42	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	05-Jun		
43	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	16-Jul		
48	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		04-Jun	05-Jun		
49	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	08-Jun		
50	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	11-Jun		
52	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	15-Jun		
53	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun			
55	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		04-Jun	23-Jun		
56	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	10-Jun		
59	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	09-Jun		
61	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	12-Jun		
62	YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	22-Jun		
63	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	11-Jun		
64	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		04-Jun	16-Jun		
65	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	10-Jun		
70	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	11-Jun		
74	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	22-Jun		
78	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	05-Jun	11-Jun		
79	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	10-Jun		
80	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		05-Jun	22-Jun		
81	YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	17-Jun		
84	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	04-Jun	16-Jun		
85	YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	08-Jun	10-Jun		
86	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		11-Jun	12-Jun		
87	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	08-Jun	03-Jul		
88	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	16-Jun		
89	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		09-Jun	23-Jun		
91	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	08-Jun		
92	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	04-Jun			
94	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	01-Mar	10-Jun		
95	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	05-Jun	16-Jun		
96	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	05-Jun		
99	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		03-Jun	05-Jun		
103	YES YES YES	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Samples not tested
108	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		04-Jun	05-Jun		
138	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	05-Jun		
150	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	09-Jun	12-Jun		
154	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	08-Jun		
269	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	08-Jun	08-Jun		
270	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	10-Jun		
282	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	03-Jun	15-Jun		
314	YES YES	HH	YY		HD	CC		HH	CC		HH	CY		HH	YY		11-Jun	23-Jun		
327	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	15-Jun	17-Jun		
331	YES YES YES	HH	YY	SS	HD	CC	SS	HH	CC	SS	HH	CY	SS	HH	YY	SS	22-May	24-Jun		