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

SAMPLES 5A01-5A05/2016

DISPATCHED ON 09TH FEBRUARY 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 and melting curve analysis	Roche diagnostic	Fluorescent labeled probes	Mangasser-Stephan, K (1999), Rapid genotyping of gene mutations with fluorescent hybridisation probes. Clinical chemistry 45, No 12		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 Tech/Fisher	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	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 Mulligan et al, 1998, GUT:42, 566-569		No
35	PCR-SSP, Taqman	Life Technologies	Standard gel, Fluorescence	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-PCR			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	PCR amplification of the relevant region of DNA by M13 tagged primers followed by direct Sanger sequencing	Invitrogen	ABI 3730 genetic analyser (capillary array)			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			IVS3 + 1G/T
63	Taqman AD	Life Technologies	RT-PCR Fluorescence	RT-PCR custom primers/probes		No
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 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/Roche	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 diagnostice	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				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
269	PCR-SSP and PCR-SSOP	Euroimmun	Fluorescence			Codon 168 (E and/or X)
270	PCR-RFLP	In-house	Acrylamide gel and BET coloration			No
282	RT-PCR	Sigma Aldrich Proligo	Fluorescence			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 Applera (Applied Biosystems) and primers Sigma Aldrich	Fluorescence	Jacques B. et al. Human mutation 19:554-559, 2002		No

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RESULTS

Consensus:		HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
Lab. No.	Assessment 63 282 65	Codon 63 5A01/2016	Codon 282 5A01/2016	Codon 65 5A01/2016	Codon 63 5A02/2016	Codon 282 5A02/2016	Codon 65 5A02/2016	Codon 63 5A03/2016	Codon 282 5A03/2016	Codon 65 5A03/2016	Codon 63 5A04/2016	Codon 282 5A04/2016	Codon 65 5A04/2016	Codon 63 5A05/2016	Codon 282 5A05/2016	Codon 65 5A05/2016	Comments
1	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
4	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
5	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
13	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
14	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
15	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
17	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
19	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
22	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
33	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
34	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
35	YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
36	YES YES YES																No results returned
37	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
39	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
42	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
43	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
48	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
49	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
50	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
52	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
53	YES YES NO	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		Following the latest EMQN best practice guidelines, Porto G et al 'EMQN best practice guidelines for the molecular genetic diagnosis of hereditary hemochromatosis', Eur J Hum Genet (2015), 1-17, where neither testing nor reporting is recommended for S65C, this variant is not included in our analysis
55	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
56	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
59	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
62	YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
63	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
64	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
65	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
70	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
74	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
78	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
79	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
80	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
81	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
84	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
85	YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
86	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
88	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
89	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
91	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		5A02/2016: Codon 63 result equivocal as faint band seen on gel
92	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
94	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
95	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
96	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
97	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
99	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
108	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
138	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
150	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
154	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
269	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
270	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
282	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
314	YES YES	HD	CY		HH	CC		HH	CY		HD	CC		HH	CC		
327	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	
331	YES YES YES	HD	CY	SS	HH	CC	SS	HH	CY	SS	HD	CC	SS	HH	CC	SS	