

SAMPLES 5A01-5A05/2017

DISPATCHED ON 21ST FEBRUARY 2017

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

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 probe	Mangasser-Stephan, K (1999), Rapid genotyping of gene		No
5	Lightcycler 480 melt curve genotyping	Roche-Tib MolBio lightmixer K	Fluorescence			No
14	PCR-RFLP	Life Tech/Fisher	3% agarose ge	Feder et al 1996		No
15	PCR-SSP	In-house	Standard ge			No
17	PCR-SSP	In-house	Standard ge	Mulligan et al, GUT (1998), 42 (4), 566-568		No
19	PCR-SSP	In-house	Standard ge			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 MolBio lightmix	RT-PCR Fluorescence Roche			No
34	PCR-SSP		Standard ge	Gutteridge et al, 1997, Vox Sanguinis		No
35	PCR-SSP, Taqman	Life Technologies	Standard gel, Fluorescence	Mulligan et al, 1998, GUT.42, 566-568		No
36						No
37	PCR-RFLP	Sigma	Agarose gel, gel red fluorescence			No
39	PCR-SSP	In-house	Standard ge			No
42	PCR-SSP	Integrated DNA Technologie	Agarose gel/Safeview Stain			No
43	PCR - Melt curve analysis	Tib Bio/Roche	Fluorescence			No
48	PCR-SSP	Eurogentec	Standard ge	Gurtridge, Vox SANG 75, 1996		No
50	ARMS-PCR	MWG eurofins	Agarose gel + EB staining	Baty et al. J Clin Pathol 1996.51:73-74		No
55	PCR-SSP	Hain	Melt curve analysis - Fluorescence			No
56	Amplicon-based next generation sequencing	In-house	Illumina MiSeq			No
59	Restriction enzyme PCF	Eurofins	Standard ge			No
62	PCR-SSP	Sigma Aldrich	Standard agarose ge			IVS3 + 1G/T
64	Agena Sequenon	Metabion	Tandem-MS based assay			No
65	PCR and allelic discrimination by Taqman probe/5' nucleas	Life Technologies/Applied Biosystems	Fluorescence	NCBI dbSNP, rs1800562 and rs1799945		No
70	RT-PCR - Lightcycler 480	Tib MolBio	Fluorescence			No
74	PCR and Sequencing	Eurogentec	qPCR			No
78	Allelic discriminator	ABI	qPCR			No
79	Taqman	Sigma and Exiqon primers, IDT	Fluorescence			Full gene sequencing using NGS for HFE
80	PCR lightcycler, Melt curve analysis	Genes 4U, Ratiogen AC	Fluorescence melt curve analysis	Genes 4U C282Y + H63D/S65C toolset		No
81	Lightcycler melt analysis	Sigma Aldrich/Tib MolBio	Fluorescence	Mangasser-Stephen et al (1998) Clinical Chemistry 45(10)		No
84	PCR-Fluorogenic target-specific	Tib MolBio/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 MolBio	Melting curve			No
92	PCR lightcycler - Roche melting curve analysis	Tib MolBio, Ratiogen	Fluorescence	Mangasser assay, Genes 4U		S65
94	Melt curve analysis	Metabion	LightsScanner			No
95	RT-PCR	Invitrogen/Tib	Melting curve analysis	Zhou et al (2004) Clinical Chemistry 50:1328-1331		No
96	RT-Allelic specific PCR	Euroclone diagnostic	Fluorescence			No
97	Hybridisation and simple probes used in Multiplex PCR	Tib MolBio 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 curve	Tib MolBio (Roche)	Fluorescence	Tib MolBio synthese (Roche) detection of HFE mutations		No
150	Sequencing	In-house				Exon 2 and Exon 4
154	PCR-SSO	Commercial	Hybridation on membrane			V53M, V59M, H63H, Q127H, P160delC, E168Q, E168X, W169X, Q283P, E60X, M172K, Y250X, AVAQ594-597del, N144H, V162del Codon 168 (E and/or X)
269	PCR-SSP and PCR-SSOP	Euroimmun	Fluorescence			No
270	PCR-RFLP	In-house	Acrylamide gel and BET coloration			No
282	RT-PCR	Sigma Aldrich Prolig	Fluorescence			No
288	HRM followed by sequencing of pos samples	In-house	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
344	PCR and Hybridation reverse	Commercial	Strip hybridation	ViennaLab Hemochromatosis srtipAssay B ref. V-4210		No
369	PCR-RFLP	Eurogentec	Polyacrylamide gel electrophoresis	Primers for C282 mutation : Forward 5'-tgg caa ggg taa aca gat cc-3' Reverse 5'-ctc agg cac tcc tct caa cc-3' Primers for H63D mutation : Forward 5'-aca tgg tta agg oct gtt gc-3' Reverse 5'-gcc aca tct ggc ttg aaa tt-3'		No

NEQ-125

Issue 2

UK NEQAS for H&I is operated by Velindre NHS Trust, a UKAS proficiency testing provider No 8351.

P 1 of 2

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RESULTS

Consensus:				HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS	Comments
Lab No.	Assessment 63 282 65	Codon 63 5A01/2017	Codon 282 5A01/2017	Codon 65 5A01/2017	Codon 63 5A02/2017	Codon 282 5A02/2017	Codon 65 5A02/2017	Codon 63 5A03/2017	Codon 282 5A03/2017	Codon 65 5A03/2017	Codon 63 5A04/2017	Codon 282 5A04/2017	Codon 65 5A04/2017	Codon 63 5A05/2017	Codon 282 5A05/2017	Codon 65 5A05/2017			
1	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
4	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
5	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
14	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
15	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
17	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
19	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
22	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
33	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
34	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
35	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
36	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
37	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
39	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
42	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
43	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
48	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
50	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
55	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
56	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
59	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
62	YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
64	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
65	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
70	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
74	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
78	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
79	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
80	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
81	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
84	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
85	YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
86	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
88	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
89	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
92	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
94	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
95	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
96	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
97	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
99	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
108	YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
138	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
150	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
154	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
269	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
270	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
282	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
288	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
314	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				
327	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
331	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
344	YES YES YES	HD	CC	SS	HD	CY	SS	HD	CC	SS	HH	CC	SS	HH	CC	SS			
369	YES YES	HD	CC		HD	CY		HD	CC		HH	CC		HH	CC				