

SAMPLES 5A06-5A10/2017

DISPATCHED ON 10TH OCTOBER 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-SSP	Roche diagnostic	Melting point	Mangasser-Stephan, K (1999), Rapid genotyping of gene mutations with		No
5	Lightcycler 480 melt curve genotyping	Roche-Tib MolBiol lightmixer Kit	Fluorescence			No
14	PCR-RFLP	Invitrogen	Gel	Feder et al 1996		No
15	PCR-SSP	In-house	Standard gel			No
16	PCR-Roche Lightcycler	Tib MolBiol LightMix	Fluorescence		EasyMag DNA extraction prior to analysis using Lightcycler	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
30	RT-PCR - Melting curve analysis	Tib MolBiol LightMix	RT-PCR Fluorescence			No
33	RT-PCR - Melting curve analysis	Tib MolBiol LightMix	RT-PCR Fluorescence			No
34	PCR-SSP	Life Technologies	Standard gel	Gutteridge et al, 1997, Vox Sanguinis		No
35	PCR-SSP, Taqman	Life Technologies	Standard gel, Fluorescence	Mulligan et al, 1998, GUT:42, 566-569		No
36	PCR	Roche Light Mix Tib MOLBIOL	Fluorescence			No
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 Bio/Roche	Fluorescence			No
48	PCR-SSP	Eurogentec	Standard gel	Gurtridge, Vox SANG 75, 1998		No
49	ARMS-PCR	MWG eurofins	Agarose gel + EB staining			No
55	PCR-SSP	Commercial	Fluorescence	Baty et al. J Clin Pathol 1998:51:73-74		No
56	Amplicon-based next generation	In-house	Illumina MiSeq			No
59	Restriction enzyme PCR	Eurofins	Standard gel			No
62	PCR-SSP	Sigma Aldrich	Standard agarose gel			IVS3 + 1GT
64	Agena Sequenom	Metabion	Tandem-MS based assay			No
65	PCR and allelic discrimination by Taqman	Life Technologies/Applied	Fluorescence	NCBI dbSNP: rs1800562 and rs1799945		No
70	RT-PCR - Lightcycler 480	Tib MolBiol	Fluorescence			No
74	PCR and Sequencing	Eurogentec	Fluorescence			No
78	Allelic discrimination	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 AG	Fluorescence melt curve analysis	Genes 4U C282Y + H63D/S65C toolsets		No
81	Lightcycler melt analysis	Sigma Aldrich/Tib MolBiol	Fluorescence	Mangasser-Stephan et al (1998) Clinical Chemistry 45(10), 1875-75, With		No
82	PCR-Fluorogenic target-specific	Tib MolBiol/Roche	Fluorescence			No
85	RT-PCR	In-house	Fluorescence			No
86	RT-PCR	Life Technologies	Fluorescence	Assay by design		No
88	SNP-PCR (RealTime allelic discrimination)	Applied Biosystems	RT-PCR			No
89	Lightcycler PCS melting curve	Tib MolBiol	Melting curve analysis			No
92	PCR lightcycler - Roche melting curve analysis	Tib MolBiol, Ratiogen	Fluorescence	HFE Duplex, Tib MolBiol		No S65C
94	Melt curve analysis	Own Design	LightsScanner			No
95	RT-PCR	Invitrogen/Tib	Melting curve analysis	Zhou et al (2004) Clinical Chemistry 50:1328-1335		No
96	RT-allelic specific PCR	Euroclone diagnostic	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	Fluorescence			No
108	RT-PCR	Applied Biosystems	Fluorescence			No
138	RT-PCR HFE mutation detected by fluorescent hybridization probe melting curves	Tib MolBiol (Roche)	Fluorescence	Tib MolBiol synthase (Roche) detection of HFE mutations		No
150	Sequencing	In-house				Exon 2 and Exon 4
154	PCR-SSO	Commercial	Hybridization 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	Gel			No
282	RT-PCR	Sigma Aldrich Prtiligo	Fluorescence			No
288	HRM followed by sequencing of pos samples	In-house	Fluorescence			No
314	Taqman RT-PCR	Applied biosystems	Fluorescence			No
327	PCR Hybridation Reverse RT-PCR (allelic discrimination)	Commercial Probes Applera (Applied Biosystems) and primers Sigma Aldrich	Hybridation sur bandelette	ViennaLab.ref4-220 haemochromatosis stripassay A Jacques B. et al. Human mutation 19:554-559, 2002		No
331	PCR and Hybridation reverse	Commercial	Fluorescence			No
344	PCR and Hybridation reverse	Aldrich	Strip hybridation	ViennaLab Hemochromatosis sripAssay B ref. V-4210		No
369	PCR-RFLP	Eurogentec	Commercial	Primers for C282 mutation : Forward 5'-tgg caa ggg taa aca gat cc-3' Reverse 5'-ctc agg cac tcc tcc caa cc-3' Primers for H63D mutation : Forward 5'-aca tgg tta agg cct gtt gc-3' Reverse 5'-acc aca tcc otc aa a t-3' - C282Y -Amorce sens : 5' GGC TGG ATA ACC TTG GCT GTAC 3' - Amorce antisens : 5' TCA CAT ACC CCA GAT CAC AAT GA 3' -Sonde C 282 Y sauvage 5' 6-FAM - CAG AGA TAT ACG TgC CAG G - NfQ-MGB 3' -Sonde C 282 Y mutant 5' VIC - ATA TAC GTa CCA GGT GGA G - NfQ-MGB 3' - H63D -Amorce sens : 5' GAA GCT TTG GGC TAC GTG GAT 3' - Amorce antisens : 5' ATC CCA GCC TTT CAG ACT CTG A 3' -Sonde H 63 D sauvage/V2 5' 6-FAM - TCG TGT TCT ATG ATc ATG A - NfQ-MGB 3' -Sonde H 63 D mutant V2 5' VIC - TTC GTG TTC TAT GAT gAT GA - NfQ-MGB 3'		No
376	qPCR	Own Design	Fluorescence			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

© Confidential report; no data may be published without permission from UK NEQAS for H&I

Effective Date 20/10/16

SAMPLES 5A06-5A10/2017

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

DISPATCHED ON 10TH OCTOBER 2017

RESULTS

Consensus:			HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	Date Received	Date Tested	Comments
Lab. No.	Assessment 63 282 65	Codon 63 5A06/2017	Codon 282 5A06/2017	Codon 65 5A06/2017	Codon 63 5A07/2017	Codon 282 5A07/2017	Codon 65 5A07/2017	Codon 63 5A08/2017	Codon 282 5A08/2017	Codon 65 5A08/2017	Codon 63 5A09/2017	Codon 282 5A09/2017	Codon 65 5A09/2017	Codon 63 5A10/2017	Codon 282 5A10/2017	Codon 65 5A10/2017				
1	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		11-Oct	16-Oct		
4	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
5	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
14	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		11-Oct	16-Oct		
15	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
16	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
17	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
19	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		12-Oct	13-Oct		
22	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	12-Oct	17-Oct		
33	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
34	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
35	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		11-Oct	12-Oct		
36	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	13-Oct	23-Oct		
37	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
39	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
42	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
43	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
48	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
50	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		13-Oct	17-Oct		
55	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		11-Oct	18-Oct	For routine PCR tests in Genetics, the turn around time set by the professional body (ACGS) is 4 weeks, only 2 weeks was allowed for testing the EQA samples.	
56	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
59	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		12-Oct	16-Oct		
62	YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
64	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
65	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		11-Oct	18-Oct		
70	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	11-Oct	20-Oct		
74	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	11-Oct	17-Oct		
78	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
79	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		12-Oct	16-Oct		
80	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		13-Oct	17-Oct		
81	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	12-Oct	17-Oct		
82	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		11-Oct	17-Oct		
84	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
85	YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	11-Oct	19-Oct		
86	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
88	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		11-Oct	17-Oct		
89	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
92	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
94	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	11-Oct	13-Oct	5A10/2017 codon 63 and 65 results inferred, not directly tested as YY.	
95	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
96	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		12-Oct	18-Oct		
99	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		12-Oct	19-Oct		
108	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY					
138	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
150	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
154	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	11-Oct	19-Oct		
269	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
270	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	10-Oct	17-Oct		
282	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
288	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
314	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		20-Oct	23-Oct		
327	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	16-Oct	18-Oct		
331	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS				
344	YES YES YES	HH	CY	SS	HD	CC	SS	HD	CC	SS	HH	CC	SS	HH	YY	SS	11-Oct	16-Oct		
369	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		11-Oct	17-Oct		
376	YES YES	HH	CY		HD	CC		HD	CC		HH	CC		HH	YY		16-Oct	23-Oct		