UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

The accuracy of HFE Testing in UK NEQAS for H&I's External Quality Assessment Scheme 2011-2017

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Introduction

UK NEQAS for H&I offers 19 external quality assessment (EQA) schemes to over 350 participants worldwide, including schemes for hereditary haemochromatosis (HH). HH is a disorder of iron metabolism leading to enhanced iron absorption, which over time can cause damage to organs, especially the liver.

Several mutations in the HFE gene, located near the HLA genes on chromosome 6p, are associated with HH. The majority of patients with HH are homozygous for the p.C282Y (cysteine282tyrosine) variant, but p.H63D (hisidine63aspartic acid) and p.S65C (serine65cysteine) mutations have also been implicated.

UK NEQAS for H&I have operated a HFE external quality assessment (EQA) scheme since 1999. Here we report the results of HFE testing from 2011-2017.

EQA Scheme Design

10 undisclosed blood samples are distributed to participants each year. Participants may register for the assessment of C282Y +/- H63D +/- S65C. Participants report results using the single letter amino acid code for each codon.

Results reported by at least 75% of participants are taken as the consensus results for assessment.

Laboratories failing to report the consensus findings on one or more samples are considered as 'unacceptable performers'

EQA Scheme Findings

The number of participant laboratories varied from 51-58 between 2011-2017. Most (n=37) laboratories took part in the scheme for the whole 7 year period.

All labs tested for C282Y and H63D while 21-29 also reported results for S65C.

The majority of labs tested using PCR-SSP, real time PCR, melt curve analysis or PCR-RFLP although other techniques (e.g. SBT) were also used.

Table 1: Number of 3-codon genotypes distributed 2011-2017

Codon 282	Codon 63	Codon 65	No. of Samples
CC	HD	SS	14
CY	HD	SS	6
CC	HH	SS	28
CY	HH	SS	4
YY	HH	SS	16
CC	DD	SS	4
CC	HH	SC	2

The 70 samples distributed included 7 different 3-codon genotypes (Table 1). 1-3 samples per year were homozygous for the C282Y mutation.

EQA Scheme Results

All samples reached the 75% consensus level for assessment. A total of 9582 results were assessed over the 7 year period (3940 for codon 282, 3924 for codon 63 and 1718 for codon 65).

There were 22 errors involving 15 different labs indicating an overall accuracy rate of 99.8%. There were 9 errors for codon 282, 8 errors for codon 63 and 5 for codon 65 (Table 2).

3 of the codon 282 errors incorrectly assigned samples as homozygous for the C282Y mutation and 4 errors missed samples homozygous for C282Y.

Table 2: EQA sample errors 2011-2017

Codon	Lab Report	Consensus Result	No of Reports
63	DD	HH	1
	DD	HD	2
	HD	DD	1
	HH	DD	2
	HH	HD	2
282	CC	CY	1
	CC	YY	2
	CY	CC	1
	CY	YY	2
	YY	CC	3
65	CC	SS	4
	SS	SC	1

The number of errors had shown a decreasing trend from 6 in 2011/2012 to 0 in 2015, however there were 5 errors in 2017 (Table 3).

Table 2: Number of errors per year

	2011	2012	2013	2014	2015	2016	2017
No. of Errors	6	6	1	2	0	2	5

The cause of the incorrect results were explained by laboratories for 21/22 errors; 11 were due to transcription errors, 8 due to technical incidents (e.g. primer failures, gel loading errors) and 2 errors in 2017 were due to a sample mix up involving 2 EQA samples.

Comment

Although the overall accuracy rate for HFE mutation testing is >99% the errors detected emphasise the need for ongoing participation in EQA schemes to help ensure high quality testing and accurate results.

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