Author: *Etain O'Rourke*

Approved By: *Michael Louw, Loretto Pilkington, Patrick Hayden, JS Charles*

Title: Primary Sample Manual: Molecular Biology – Haematology

Changes made since previous version: *Removed statement regarding PSM being dynamic and subject to change. Added reason for rejection for HFE genetic testing: patients under 16 years of age are not tested as per HSE guidelines. References for HSE and international guidelines included. Changed test name from 'Haemochromatosis' to 'HFE Genetic Testing'. Removed previous point 3 from 'Reasons for rejection of sample', as it was a copy of point 2. Changed 'Reference Range' to 'Results'. Reworded 'Stability' section for HFE. Added section on Laboratory Sample Storage. Split HFE results into two columns, one for C282Y and one for H63D. Added reference source for results.*

Note: *Please refer to the document record on Q-Pulse for the revision history of this document*

CONTENTS:

INTRODUCTION

This is a list of the Molecular haematology tests performed at Eurofins Biomnis' Dublin Laboratory. For a searchable list of tests performed by Eurofins Biomnis in France, in our laboratories in Lyon and Paris, click here.

Please contact our Client Services department on Free Phone 1800-252-966 or 01 295 8545, or e-mail clientservices@ctie.eurofinseu.com with any questions.

For sample collection, please contact our Logistics department on Free Phone 1800-252-967, or e-mail logistics@ctie.eurofinseu.com.

TEST INFORMATION TEMPLATE	
Brief information on clinical background, indications for test and interpretation of test results.	
Preparation of Patient: any special preparation required, such as fasting. **Precautions:** any special circumstances, conditions etc. to be aware of.	
Accredited	Whether or not the test is accredited by INAB to ISO 15189. If the test is accredited (Yes), this section is colour-coded in green; if the test is not accredited (No), this section is colour-coded in orange.
Method	Test method. Standard Operating Procedure reference for this test.
Sample Requirements	Type of tube required, transport temperature and other information.
Turnaround Time	The maximum turnaround time in working days from receipt of the sample in the laboratory's pre-analytics department to the authorisation of the result. **Working days are Monday to Friday 08:00 to 18:00.**
Stability	Sample stability under various conditions. **RT = room temperature i.e.: 16 – 25 °C.** Please see **SAMPLE STABILITY** notes below. Stability data indicated by a superscript numeral 1 are taken from the publication referenced below[1].
Laboratory Sample Storage	The length of time that the laboratory stores patient samples for and the conditions in which the samples are stored.
Results and Source	The potential results that may be released for the test. Source of the results.

NOTES ON SAMPLE STABILITY

Most incorrect laboratory test results are due to improper sample collection and transport. For details regarding correct phlebotomy technique and our patient identification requirements, please click here.

In order for you to arrange and properly time phlebotomy and sample collection, we have indicated, for each test, its stability after collection.
Stability data are taken from the reference literature indicated below[1].

REFERENCE

1. Permenter, J., Ishwar, A., Rounsavall, A., Smith, M., Faske, J., Sailey, C.J., Alfaro, M.P. (2015). Quantitative analysis of genomic DNA degradation in whole blood under various storage conditions for molecular diagnostic testing. *Molecular and Cellular Probes*, 29(6):449-453.

2. HSE National Laboratory Handbook: Laboratory Testing for Hereditary Haemochromatosis. Document reference number CSP035/2019 version 1.

3. Porto, G., Brissot, P. Swinkels, D.W., Zoller, H., Kamarainen, O., Patton, S., Alonso, I., Morris, M., Keeney, S. (2016). EMQN best practice guidelines for the molecular genetic diagnosis of hereditary haemochromatosis (HH). *European Journal of Human Genetics*, 24:479-495.

REASONS FOR REJECTION OF SAMPLES/NON-REPORTING OF TESTS

1. Samples received beyond the stability limits and/or not at the correct temperature indicated below for each test.

2. Samples received in the incorrect tube/with the incorrect anticoagulant or lack of the correct anticoagulant.

3. Samples received for HFE Testing for patients under 16 years of age. HFE genetic testing is not performed on patients under the age of 16, in accordance with HSE and International guidelines.

HFE GENETIC TESTING

Haemochromatosis is an autosomal recessive disorder of iron metabolism that affects approximately 0.2-0.5% of the Caucasian population, with a higher-than-average incidence in the Irish population. The disease is characterized by the excessive accumulation of iron in the body and caused by an increased absorption of dietary iron at the intestinal mucosa level.

The HFE gene is responsible for the disease and was identified in 1996 (Feder JN et al., 1996); it is localized on the short arm of chromosome 6, near the locus of the HLA-A gene. A mutation of this gene causes the synthesis of an abnormal protein unable to interact with the transferrin receptors, favoring the transport of iron through the intestinal mucosa. However, the exact mechanism with which the mutated HFE protein contributes to the increased intestinal absorption is not completely clear.

The two most frequent mutations found in the HFE gene correspond to the C282Y mutation (substitution of a cysteine with a tyrosine in position 282 of the protein) and the H63D mutation (substitution of the histidine with an aspartic acid in position 63 of the protein).

Homozygosity for C282Y accounts for 90% of haemochromatosis. The clinical penetrance (i.e., the risk of developing iron overload disease) in C282Y homozygous patients has been reported as being between 60 and 90% but the risk is not well defined.
Patients heterozygous for either the C282Y or H63D mutation have no significant risk of developing hemochromatosis but are carriers and as such should receive genetic counselling.
Compound heterozygotes (one copy of each of the C282Y and H63D mutations) have a very low risk of developing iron overload (0.5 to 1%).
Homozygous H63D patients also have a very low risk of developing iron overload (less than 0.2%).

Preparation of patient: There is no physical preparation for the haemochromatosis test.

Precautions: None.

Accredited	Yes	
Method	Molecular Biology - Real time PCR SOP: MB55	
Sample Requirements	Tube Type: Whole blood EDTA Temperature: 2-8°C Miscellanous: Non fasting	
Turnaround Time	7 working days from sample receipt.	
Stability	Whole blood in EDTA at room temperature: 3 days Whole blood in EDTA at 2-8°C: 1 year DNA extracts at -30°C to -15°C: Long-term. No specific time frame stated.	
Laboratory Sample Storage	Primary sample tube (whole blood in EDTA): 3 months at 2-8°C DNA extracts: 3 months at -20°C	
Results and Source	Homozygous for C282Y Heterozygous for C282Y C282Y mutation not present.	Homozygous for H63D Heterozygous for H63D H63D mutation not present.

	ViennaLab HFE mpx RealFast™ Assay Instructions For Use version 12/2020

Consultant Responsible: Dr. Patrick Hayden
Eurofins Biomnis: +353-1-295-8545