Frequently Asked Questions: ***HFE*-Hereditary Hemochromatosis (*HFE*-HH) Genetic Testing**

Frequently asked questions addressed in this document:

See disease-specific page at www.genebc.ca for other relevant information.

1. Why is testing for the H63D variant not available in BC?

The risk of clinical disease associated with H63D is low in the absence of predisposing risk factors, for example, alcohol abuse[1,2]. The identification of this variant is not informative for clinical management, i.e., decisions regarding further testing, or treatment, are unaffected by the presence or absence of the H63D variant*; thus, this testing is not recommended and is no longer available in BC.

*A given clinician may utilize the presence or absence of H63D as a discriminator on which to base clinical management decisions. But as there is not a scientific rationale for doing so, such use would be considered an over-interpretation of this genetic information.

Supporting information:

- *HFE*-HH is one of many causes of elevated ferritin levels[3]. The *most likely* cause of elevated ferritin levels in individuals who have a genotype of C282Y;H63D or H63D;H63D are *clinical* risk factors such as inflammation, alcohol consumption and obesity.
- The H63D variant is approximately twice as common in the Northern European general population as the C282Y variant. In the Northern European general population, approximately 0.8% (1/120) of individuals are compound heterozygotes for C282Y and H63D and 2.1% (1/48) of individuals are homozygous for H63D[4].
- Individuals who are C282Y;H63D compound heterozygotes may demonstrate ***biochemical*** hemochromatosis (see definition below); however, these individuals are highly unlikely to present with ***clinical*** manifestations of this disorder in the absence of secondary clinical risk factors[5-7].
- Individuals homozygous for the H63D variant (H63D;H63D) may show elevated ferritin or transferrin saturation levels but this genotype has not been associated with clinically significant iron overload in the absence of predisposing risk factors, for example, alcohol abuse[1,2,5].

Definitions:

Biochemical hemochromatosis: Evidence of iron overload as demonstrated by elevated transferrin saturation (TSAT>0.45%); supported by elevated ferritin (>200 µg/L in women; >300 µg/L in men). Biochemical hemochromatosis is usually present before clinical expression of the disease[8].

Clinical hemochromatosis: Clinical symptoms of iron overload. Clinical findings are supported by documented biochemical hemochromatosis[8]. For more information, see bcguidelines.ca High Ferritin and Iron Overload – investigation and management.

2. How do I order *HFE*-HH genetic testing to confirm a previous genetic diagnosis?

Clinical Scenario: Recently phlebotomized patient reports a previous genetic diagnosis *HFE*-HH; genetic report not available; does not meet chemistry criteria for *HFE*-HH genetic testing.

WHAT TO DO:

- **Confirm patient is of European Ancestry****
- **Use Standard Outpatient Laboratory Requisition (SOPLR)**
  - https://www2.gov.bc.ca/assets/gov/health/forms/1901fil.pdf
  - See Appendix for example of completed requisition
- **Write the following in the indicated section*****:
  - Diagnosis section: **Previous *HFE*-HH genetic diagnosis & treated by phlebotomy**
  - Other Tests section: ***HFE*-HH genetic testing**

** Note: "DNA testing" includes only the C282Y variant, which is very rare in individuals who are not of European ancestry. How to order testing for other genetic causes of hemochromatosis is outside the scope of this FAQ.

*** Both sections must include the indicated language. Otherwise, the laboratory may cancel the test or request further information, depending on the information provided on the requisition.

WHAT NOT TO DO:

- DO NOT use *HFE*-hemochromatosis Confirmation of Diagnosis (ferritin first, ±TS, ± DNA testing)
  - Rationale: If patient does not meet chemistry requirements, then *HFE*-HH genetic testing will not be performed.
- DO NOT use *HFE*-hemochromatosis Sibling/Parent is C282Y/C282Y homozygote (DNA testing)
  - Rationale: Reports will have the incorrect interpretation.

3. Why did *HFE*-HH genetic testing NOT confirm my patient's previous *HFE*-HH genetic diagnosis?

- The *HFE*-HH assay tests for the presence of the C282Y variant only.
- Apparent heterozygosity for the C282Y variant may mean that the patient is a compound heterozygote for C282Y and a second HFE variant, or it may mean that the patient was treated by phlebotomy based on heterozygosity and other clinical findings/diagnostic testing without confirming compound heterozygosity.
- An apparently negative result may mean that the patient was treated by phlebotomy without confirming a diagnosis of *HFE*-HH. Or, the patient may be homozygous for an *HFE* variant not detected by the assay; in such cases it is most likely the patient was treated based on homozygosity for H63D (not generally an indication for phlebotomy).

Appendix

BRITISH COLUMBIA | Ministry of Health

STANDARD OUT-PATIENT LABORATORY REQUISITION

ORDERING PRACTITIONER: ADDRESS, PHONE, MSP PRACTITIONER NUMBER

Yellow highlighted fields must be completed.

For tests indicated with a blue tick box, consult provincial guidelines and protocols (www.BCGuidelines.ca)
https://www2.gov.bc.ca/gov/content/health/practitioner-professional-resources/bc-guidelines

Bill to → ☐ MSP ☐ ICBC ☐ WorkSafeBC ☐ PATIENT ☐ OTHER: _____

PERSONAL HEALTH NUMBER | ICBC/WorkSafeBC NUMBER | LOCUM FOR PRACTITIONER AND MSP PRACTITIONER NUMBER:

LAST NAME OF PATIENT | FIRST NAME OF PATIENT | If this is a STAT order please provide contact telephone number:

DOB YYYY MM DD | SEX ☐ M ☐ F | Pregnant? ☐ YES ☐ NO ☐ Fasting?_____h pc | Copy to PRACTITIONER/MSP Practitioner Number:

PRIMARY CONTACT NUMBER OF PATIENT | SECONDARY CONTACT NUMBER OF PATIENT | OTHER CONTACT NUMBER OF PATIENT | Copy to PRACTITIONER/MSP Practitioner Number:

ADDRESS OF PATIENT | CITY/TOWN | PROVINCE | POSTAL CODE

DIAGNOSIS

Previous HFE-HH genetic diagnosis & treated by phlebotomy

CURRENT MEDICATIONS/DATE AND TIME OF LAST DOSE

HEMATOLOGY

- ☐ Hematology profile
- ☐ INR
- ☐ Ferritin (query iron deficiency)

On Anticoagulant? ☐ Yes ☐ No
Specify: _____

HFE - Hemochromatosis (check ONE box only)
- Confirm diagnosis (ferritin first, ±TS, ±DNA testing)
- Sibling/parent is C282Y/C282Y homozygote (DNA testing)

URINE TESTS

- Macroscopic → microscopic if dipstick positive
- Macroscopic → urine culture if pyuria or nitrite present
- Macroscopic (dipstick) Microscopic *

* Clinical information for microscopic required:

_____

CHEMISTRY

- ☐ Glucose – fasting (see reverse for patient instructions)
- ☐ Glucose – random
- ☐ GTT – gestational diabetes screen (50 g load, 1 hour post-load)
- ☐ GTT – gestational diabetes confirmation (75 g load, fasting, 1 hour & 2 hour test)
- ☐ GTT – non-gestational diabetes
- ☐ Hemoglobin A1c
- ☐ Albumin/creatinine ratio (ACR) - Urine

MICROBIOLOGY – LABEL ALL SPECIMENS WITH PATIENT'S FIRST & LAST NAME, DOB, PHN & SITE

ROUTINE CULTURE

On Antibiotics? ☐ Yes ☐ No Specify: _____

☐ Throat ☐ Sputum ☐ Blood ☐ Urine

☐ Superficial Wound, Site: _____

☐ Deep Wound, Site: _____

☐ Other: _____

VAGINITIS

- ☐ Initial (smear for BV & yeast only)
- ☐ Chronic/recurrent (smear, culture, trichomonas)
- ☐ Trichomonas testing

GROUP B STREP SCREEN (Pregnancy only)

☐ Vagino-anorectal swab ☐ Penicillin allergy

CHLAMYDIA (CT) & GONORRHEA (GC) by NAAT

Source/site: ☐ Urethra ☐ Cervix ☐ Urine ☐ Vagina ☐ Throat ☐ Rectum

Other _____

GONORRHEA (GC) CULTURE

Source/site: ☐ Cervix ☐ Urethra ☐ Throat ☐ Rectum

Other _____

STOOL SPECIMENS

History of bloody stools? Yes

C.difficile testing | Stool culture | Stool ova & parasite exam

Stool ova & parasite (high risk, submit 2 samples)

DERMATOPHYTES

☐ Dermatophyte culture ☐ KOH prep (direct exam)

Specimen: ☐ Skin ☐ Nail ☐ Hair

Site: _____

MYCOLOGY

☐ Yeast ☐ Fungus Site: _____

HEPATITIS SEROLOGY

Acute viral hepatitis undefined etiology
Hepatitis A (anti-HAV IgM)
Hepatitis B (HBsAg + anti-HBc)
Hepatitis C (anti-HCV)

Chronic viral hepatitis undefined etiology
Hepatitis B (HBsAg; anti-HBc; anti-HBs)
Hepatitis C (anti-HCV)

Investigation of hepatitis immune status
Hepatitis A (anti-HAV, total)
Hepatitis B (anti-HBs)

Hepatitis marker(s)
HBsAg
(For other hepatitis markers, please order specific test(s) below)

☐ HIV Serology
(patient has the legal right to choose not to have their name and address reported to public health = non-nominal reporting)
☐ Non-nominal reporting

OTHER TESTS – Standing Orders include expiry & frequency

- ☐ ECG
- ☐ FIT (Age 50-74 asymptomatic q2y) Copy to Colon Screening Program
- ☐ FIT No copy to Colon Screening Program

HFE-HH genetic testing

LIPIDS

☑ one box only

Note: Fasting is not required for any of the panels but clinician may specifically instruct patient to fast for 10 hours in select circumstances (e.g. history of triglycerides > 4.5 mmol/L), independent of laboratory requirements.

- Full Lipid Profile - Total, HDL, non-HDL, LDL cholesterol, & triglycerides (Baseline or Follow-up of complex dyslipidemia)
- Follow-up Lipid Profile - Total, HDL & non-HDL cholesterol only
- Apo B (not available with lipid profiles unless diagnosis of complex dyslipidemia is indicated)

THYROID FUNCTION

For other thyroid investigations, please order specific tests below and provide diagnosis.

- Monitor thyroid replacement therapy (TSH Only)
- Suspected Hypothyroidism (TSH first, fT4 if indicated)
- Suspected Hyperthyroidism (TSH first, fT4 & fT3 if indicated)

OTHER CHEMISTRY TESTS

- ☐ Sodium
- ☐ Potassium
- ☐ Albumin
- ☐ Alk phos
- ☐ ALT
- ☐ B12
- ☐ Bilirubin
- ☐ GGT
- ☐ T. Protein
- ☐ Creatinine / eGFR
- ☐ Calcium
- ☐ Creatine kinase (CK)
- ☐ PSA – Known or suspected prostate cancer (MSP billable)
- ☐ PSA screening (self-pay)
- ☐ Pregnancy test
- ☐ ß-HCG – quantitative

SIGNATURE OF PRACTITIONER | DATE SIGNED

DATE OF COLLECTION | TIME OF COLLECTION | COLLECTOR | TELEPHONE REQUISITION RECEIVED BY: (employee/date/time)

INSTRUCTIONS TO PATIENTS (See reverse)
Other Instructions:

The personal information collected on this form is collected under the authority of the *Personal Information Protection Act*. The personal information is used to provide medical services requested on this requisition. The information collected is used for quality assurance management and disclosed to healthcare practitioners involved in providing care or when required by law. Personal information is protected from unauthorized use and disclosure in accordance with the *Personal Information Protection Act* and when applicable the *Freedom of Information and Protection of Privacy Act* and may be used and disclosed only as provided by those Acts.

References

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3 Adams, P. C. *et al.* HFE mutations in Caucasian participants of the Hemochromatosis and Iron Overload Screening study with serum ferritin level <1000 microg/L. *Can J Gastroenterol* **27**, 390-392, doi:10.1155/2013/493170 (2013).

4 Karczewski, K. J. *et al.* The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* **581**, 434-443, doi:10.1038/s41586-020-2308-7 (2020).

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