Guidelines for the Use of Laboratory Tests for Assessment of Iron Overload (CLP 001)

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I. Purpose

To provide clinicians with a concise reference document describing the appropriate laboratory tests for the assessment of iron overload in adult patients.

Readers are reminded that OAML Guidelines will not apply to every clinical situation, nor can they serve as a substitute for sound clinical judgment.

2. Background

Iron overload disorders are characterized by the abnormal accumulation of iron in the body.

The early detection of iron overload is important due to the toxic and often irreversible effects of excess iron on various organs.

Iron overload can occur as a result of inherited or secondary causes. Hereditary hemochromatosis (HH) is the most common autosomal recessive genetic disorder in persons of Northern European descent. It occurs with a prevalence of approximately 1 in 200-500 individuals. Classic hereditary hemochromatosis is the result of a mutation in the *HFE* gene on chromosome 6, typically C282Y or H63D. However, the clinical manifestations of hemochromatosis are observed in less than 10 per cent of those who carry the mutation. Homozygous carriers are at greatest risk of increased absorption of dietary iron that may culminate in severe damage to multiple organs. Other less common non-*HFE* mutations also exist with a range of clinical consequences.

There are a variety of distinct syndromes of secondary iron overload that need to be clinically distinguished from hereditary hemochromatosis. Examples of these conditions include anemia from ineffective erythropoiesis, chronic hemolytic anemia, various liver diseases, excessive ingestion of medicinal iron, and chronically transfused patients. Examples of hereditary and secondary causes of iron overload are listed in Table 1.

Table 1: Causes of Iron Overload and Examples of Associated Clinical Conditions

Causes	Examples
1.Inherited causes of iron overload	Hereditary hemochromatosis "*HFE* and non-*HFE* mutations"
2.Secondary iron overload	Parenteral or accidental iron overload Chronic red cell transfusion Thalassemia major G6PD deficiency Pyruvate kinase deficiency Sideroblastic anemia **Chronic liver disease • Hepatitis B or C, • alcoholic cirrhosis, • non-alcoholic steatohepatitis • Porphyria Cutanea Tarda
3. Miscellaneous	Congenital atransferrinemia

NOTE: This list is not exhaustive.
**CAUTION: These conditions often lead to elevated ferritin levels due to inflammation unrelated to iron overload.

3. Indications for Iron Overload Screening

Although most reviews have concluded that there is insufficient evidence at this point to warrant general population screening for iron overload, there is widespread consensus that efforts to increase early detection and treatment of hemochromatosis are warranted.

Because iron overload can adversely affect several organ systems, its symptoms can be confused with those of more common diseases, such as alcoholic liver disease, diabetes, and osteoarthritis. Years before organ dysfunction resulting from iron overload becomes apparent, non-specific symptoms such as arthralgias, fatigue, and abdominal pain may be observed.

Patients with unexplained signs and symptoms possibly related to iron overload should be screened. These signs and symptoms include:

- arthritis,
- persistent elevation of liver enzymes or cirrhosis,
- adult-onset diabetes,
- congestive heart failure,
- male sexual dysfunction (secondary hypogonadism),
- increased skin pigmentation,
- persistent elevation of serum ferritin levels not explained by inflammation or systemic disease.

Asymptomatic at-risk patients should also be screened. These include:

- first degree relatives of patients with confirmed hemochromatosis
- patients at risk of iron overload due to a secondary underlying condition, for example:
    - iron loading anemias,
    - chronic transfusion.

4. Testing Recommendation for Iron Overload Investigation

The appropriate tests to use in the investigation of iron overload are Serum Ferritin (SF) and Percent Transferrin Saturation (TS %). Rather than relying on the result of only one of these tests, several sources [4,7,9,11] recommend using the results of both these tests in combination to increase the predictive accuracy of an iron overload diagnosis (see Table 2 below).

Table 2: Iron Overload Investigations and Application
(Applicable to adults ≥18 years of age and ≥100 lbs.)

Investigation	Application
normal TS%* and SF**	No further testing is required.
TS% >45% and normal SF	Repeat test results in one month If repeat TS% >45%, consider *HFE* genotyping
TS% >45% for women and >50% for men and SF increased	*HFE* genotyping is recommended (see section 5)
TS% normal and SF increased	Exclude inflammatory causes for ferritin elevation. If inflammation is excluded, consider *HFE* genotyping.

***TS% has wide biologic variation; repeat testing for confirmation of elevated TS% is recommended before further testing (i.e. DNA testing). Fasting prior to a blood draw for TS% is not necessary, since it has not been shown to reduce biologic variation.

****SF typically has a wide reference range and varies with age and gender – consult your laboratory report for your laboratory's reference range.

Liver function testing is also recommended in the general assessment of patients with suspected or documented iron overload, specifically in patients with a serum ferritin >800 µg/L.

HFE genotyping should be reserved for confirmatory testing of hereditary hemochromatosis (see section 5).

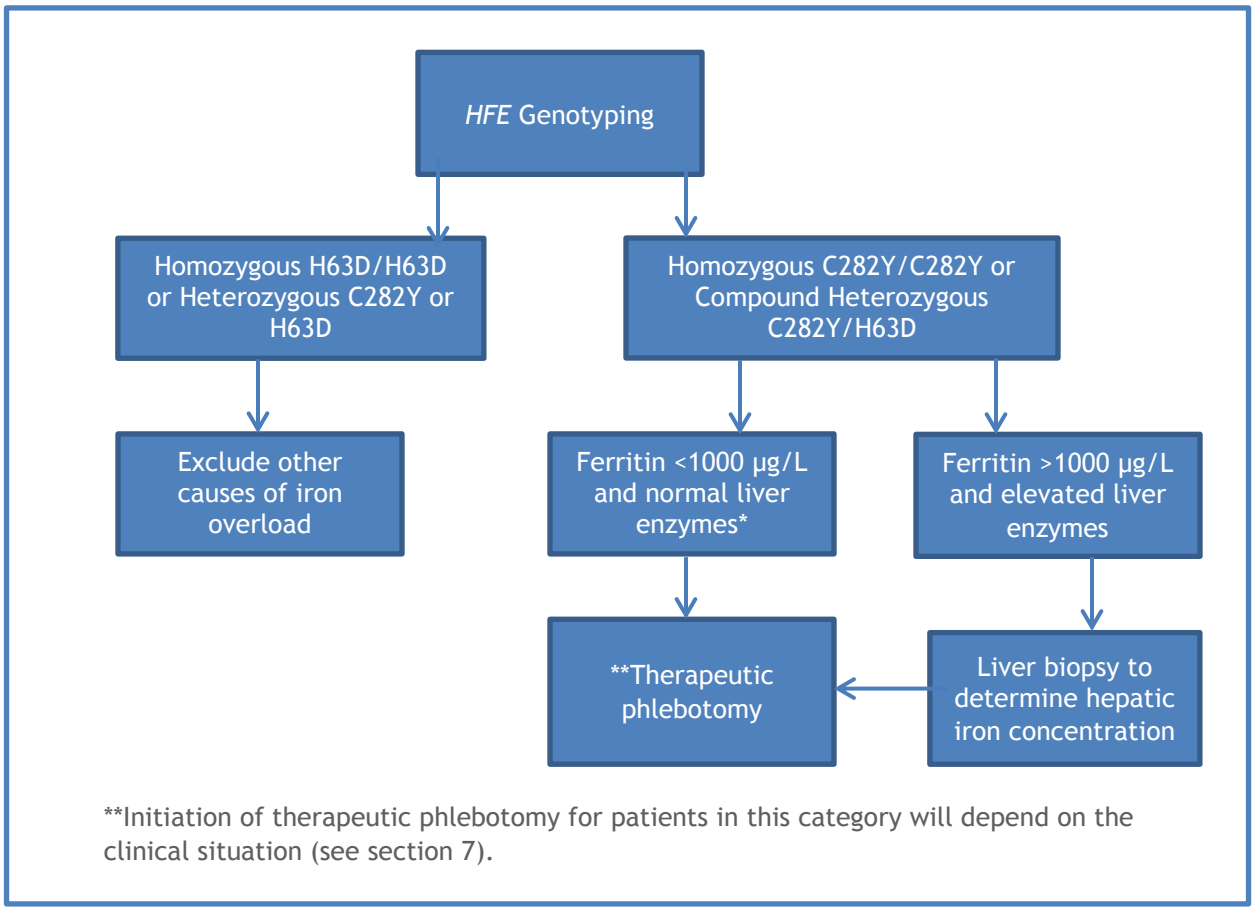
5. *HFE* genotyping for Hereditary Hemochromatosis

HFE genotyping should be offered to those patients meeting the criteria outlined in Table 2, or to those patients with first degree relatives with documented hemochromatosis. The possible results and implications for positive gene mutation testing are described in Table 3. An algorithm for testing and treatment of HH is seen in Figure 1.

Table 3: Possible Results and Implications for Positive Gene Mutation Testing

Genotype	Phenotype
Homozygous C282Y/C282Y	60-90% of patients with this genotype will develop clinical iron overload
Compound Heterozygous C282Y/H63D	Significant iron overload occurs in up to 10% of these individuals
Homozygous H63D/H63D	Up to 4% of patients with this genotype develop clinical iron overload
Heterozygous C282Y/WT (wild type) or H63D/WT	It is rare for patients with this genotype to develop iron overload

Figure 1: Algorithm for Testing and Treatment of HH*



*Algorithm for Testing and Treatment of HH obtained from 2011 American Association for the Study of Liver Diseases Practice Guideline: Diagnosis and Management of Hemochromatosis

Patients testing positive for homozygous C282Y mutations, compound heterozygotes, or those with iron overload not explained by DNA results should be referred to a specialist for genetic counseling and/or treatment.

The non-*HFE* forms of inherited iron overload are rare, accounting for <5% of cases encountered. Genetic testing for these mutations is largely unavailable except in research laboratories. Screening for non-*HFE* mutations is not recommended.

With *HFE* genotyping readily available, liver biopsy is performed less frequently for diagnosis of hereditary hemochromatosis. In general, liver biopsy is performed to investigate cirrhosis in patients with elevated liver enzymes or serum ferritin levels >1000 µg/L.

6. Laboratory Requisitions for *HFE* Genotyping

Requisitions for *HFE* genotyping should be obtained from the institution nearest you that provides the testing. The following is a list of laboratories offering *HFE* genotyping for hemochromatosis and websites where the requisitions can be downloaded:

Sites with Specific Procedures for Ordering DNA	Requisition
London Health Sciences Centre	http://www.lhsc.on.ca/lab/molegen/requ.pdf
Children's Hospital of Eastern Ontario	http://www.cheo.on.ca/uploads/genetics/files/genetics_form_5549.pdf
Kingston General Hospital	http://www.path.queensu.ca/kgh/genetics/2007dnareqscan.pdf
McMaster University Medical Centre	http://www.lrc.hrlmp.ca/AttachedFiles/RequisitionForm/Requisition_for_Genetic_Testing.pdf
North York General Hospital	http://www.nygh.on.ca/data/2/rec_docs/310_Molecular_Genetics_lab_Req_form.pdf
Credit Valley Hospital	http://www.cvh.on.ca/genetics/requisitions.php Physician will need to call for access to requisition: 905-813-4104
Sunnybrook Health Sciences Centre	Physician must call Sunnybrook Hospital for verification of appropriate test and ordering instructions: 416-480-6100 ext. 89572
University Health Network - Toronto General Hospital	http://www.uhn.ca/applications/labdictionary/View.aspx?lid=1240

HFE genotyping for hemochromatosis is performed at no cost to the patient.

7. Follow-up and Treatment

Hereditary Hemochromatosis

Phlebotomy remains the mainstay of treatment for HH and if initiated before the onset of cirrhosis and/or diabetes has been shown to significantly reduce morbidity and mortality.

Patients with homozygous HH and elevated serum ferritin, or those patients whose liver biopsy showed evidence of iron overload should be treated. Asymptomatic homozygous patients without indication of iron overload should have regular monitoring for early detection of disease progression.

During treatment, serum ferritin is the recommended test for monitoring iron stores because TS% remains elevated until iron stores are depleted. The target level for ferritin should be 50-100 µg/L and iron deficiency should be avoided. Once this target ferritin level is achieved,

phlebotomy should be stopped and ferritin levels should be monitored to assess for the presence of iron re-accumulation and the need for maintenance phlebotomy.

Maintenance phlebotomy generally involves phlebotomy at 2-3 month intervals depending on the patient and not all patients with HH will need maintenance therapy.

Patients in the maintenance phase of treatment are eligible to become voluntary blood donors. For more information, contact Canadian Blood Services.

Patients with cirrhosis should also be regularly screened for hepatocellular carcinoma (HCC) even after treatment as part of ongoing maintenance.

Hereditary Hemochromatosis Treatment Algorithm:

1. Previously untreated cases usually start with a phlebotomy program consisting of weekly withdrawal of 500 mL of blood. The patient's hemoglobin level is determined before each planned phlebotomy and the treatment may be modified or postponed, or even stopped if anemia develops. Cumulatively, the phlebotomy program should not reduce the patient's hemoglobin level by more than 20% of their starting hemoglobin concentration.

2. A target ferritin value of 50-100 µg/L should be used to monitor iron depletion. Generally, levels are monitored monthly, or after 4-6 phlebotomies, but this can vary depending on the clinical situation. Monitoring intervals will increase as ferritin levels drop below 200 µg/L. Iron deficiency should be avoided. If the patient has elevated serum ferritin levels due to inflammatory causes, TS% should be used for monitoring.

3. Patients who have achieved adequate iron depletion should then be regularly monitored for iron re-accumulation. Generally, the patient's ferritin level is determined 6 months after cessation of phlebotomy therapy. If clinically indicated at this time, maintenance therapy is initiated. Some symptoms may be noticeably improved after treatment; these include fatigue, skin pigmentation, and abdominal pain.

   Once iron stores have been depleted, end-organ damage should be reassessed periodically. Abnormally high levels of liver enzymes may decrease. There also may be improvement in iron-induced cardiac dysfunction and blood sugar levels of diabetics may improve.

   Symptoms less responsive to treatment include arthralgias and hypogonadism. Phlebotomy will not reverse cirrhosis; however, improvement of liver fibrosis can be seen.

4. Patients with cirrhosis should be routinely screened for HCC even after treatment. For more information about HCC screening, please refer to the National Cancer Institute document on HCC screening at http://www.cancer.gov/cancertopics/pdq/screening/hepatocellular/HealthProfessional/page1/AllPages.

Secondary/Acquired Iron Overload

Porphyria cutanea tarda is the only secondary iron overload syndrome in which phlebotomy treatment is indicated.

Currently, there is no concrete evidence supporting phlebotomy therapy for iron overload secondary to alcoholic liver disease, non-alcoholic fatty liver disease, or Hepatitis C.

Iron overload secondary to ineffective erythropoiesis or chronic hemolytic anemia is generally treated using iron chelators. Iron chelation can be achieved using deferasirox (Exjade®) or deferoxamine mesylate. A liver biopsy can potentially be used to monitor the effectiveness of treatment.

Monitoring patients with secondary iron overload is challenging. Several conditions that cause secondary iron overload also cause inflammation possibly leading to elevated serum ferritin levels that are unrelated to iron stores. Therefore, in contrast to HH where serum ferritin reliably reflects iron burden during therapy, ferritin levels can be misleading in secondary iron overload. In some cases, a liver biopsy to monitor hepatic iron concentration may be indicated.

8. Limitations

- Ferritin alone is not a reliable screening test for iron overload. For example, ferritin levels can be elevated due to conditions other than HH and may be elevated in patients with acute and chronic inflammation, liver disease, autoimmune disorders, and some types of cancer such as Hodgkin's Disease. In these clinical situations ferritin levels would fail to reflect the body's iron stores. When using ferritin levels for investigation of iron overload or treatment assessment this information should be taken into account.
- Due to the formation of new red blood cells, glycemia determined by HbA1c levels may be underestimated for up to three months after phlebotomy.

References

1. Adams, PC. et al. Biological Variability of Transferrin Saturation and Unsaturated Iron-Binding Capacity. *The American Journal of Medicine*. 2007; 120:999.e1-999.e7.

2. Adams, PC., Barton, JC. How I treat hemochromatosis. *Blood*. 2010; 116:317-325.

3. American Society of Hematology. *American Society of Hematology Self-Assessment Program Textbook*. (Third Edition). Lancaster, PA: Cadmus Communications. 2007.

4. Bacon, RB. et al. Diagnosis and Management of Hemochromatosis: 2011 Practice Guideline by the American Association for the Study of Liver Diseases. *Hepatology*. 2011; 54:328-343.

5. Basset ML., Hickman PE., Dahlstrom JE. The changing role of liver biopsy in diagnosis and management of haemochromatosis. *Pathology*. August 2011; 43(5):433-439.

6. Brissot P., Bardou-Jacquet E., Jouanelle A-M., Loréal O. Iron Disorders of Genetic Origin: a changing world. *Trends in Molecular Medicine*. December 2011; 17(12):707-713.

7. British Columbia Guidelines and Protocols Advisory Committee. Iron Overload – Investigation and Management. December 15, 2006.

8. Duchini, A. Hemochromatosis. Medscape Reference – Drugs, Diseases, and Procedures. Available at: http://emedicine.medscape.com/article/177216-overview. (Accessed June, 2012).

9. European Association for the Study of the Liver. EASL Clinical Practice Guidelines for HFE Hemochromatosis.J Hepatol (2010). doi: 10.1016/j.jhep.2010.03.001. © 2010 European Association for the Study of the Liver. Available at: http://www.easl.eu/assets/application/files/03d32880931aac9_file.pdf. (Accessed October, 2012).

10. Fleming, R.E., Ponka P. Iron Overload in Human Disease. *NEJM*. 2012; 366:348-359.

11. Haute Autorité de santé (HAS) - Guidelines Department. Management of HFE-related haemochromatosis. July 2005. Available at: http://www.has-sante.fr/portail/upload/docs/application/pdf/hemochromatosis_guidelines_2006_09_12__9_10_9_659.pdf. (Accessed October, 2012).

12. Lewis, SM., Bain, BJ., Bates, I. *Dacie and Lewis Practical Haematology* (Tenth Edition). Philadelphia, PA: Churchill Livingstone Elsevier. 2006.

13. National Cancer Institute. Liver (Hepatocellular) Cancer Screening (PDQ®) http://www.cancer.gov/cancertopics/pdq/screening/hepatocellular/HealthProfessional/page1/AllPages. (Accessed August, 2012).

14. Pietrangelo, A. Hereditary Hemochromatosis – A New Look at an Old Disease. *NEJM*. 2004; 350:2383-2397.

15. Piperno, A. Classification and Diagnosis of Iron Overload. *Haematologica*. 1998; 83:447-455.

16. Power, TE. et al. Hemochromatosis Patients as Voluntary Blood Donors Canadian. *Journal of Gastroenterology*. 2004; 18:393-396.

17. Qaseem, A. et al. Screening for Hereditary Hemochromatosis: A clinical practice guideline from the American College of Physicians. *Annals of Internal Medicine*. 2005; 143:517-521.

18. Waalen J, Felitti VJ, Gelbart T, et al. Screening for hemochromatosis by measuring ferritin levels: a more effective approach. *Blood*. 2008; 111: 3373-3376.

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The OAML, through its Quality Assurance Committee, co-ordinates the development, dissemination, implementation and review of Guidelines for Clinical Laboratory Practice.

Guidelines are reviewed every 5 years, or as the literature warrants. When consensus on the Guideline is achieved by the Committee, the Guideline is submitted to the OAML's Board of Directors for approval before distribution to clinicians.

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