

## Molecular Testing for Alpha Thalassemia

Effective June 1, 2010, BC Biomedical Laboratories will offer a new test for alpha thalassemia using a molecular DNA assay based on a polymerase chain reaction (PCR) and reverse-dot blot hybridization. This will replace the Hemoglobin H inclusions test.

### Background

The thalassemia syndromes are the commonest inherited genetic disorder in humans. Alpha thalassemia is found predominantly in individuals of South East Asian, South Asian, African, Mediterranean and Middle East extraction but also rarely in other populations.

Human alpha globin genes are duplicated on chromosome 16, two on each chromosome, for a total complement of four alpha globin genes.

Alpha thalassemia occurs when a genetic mutation leads to a reduced production of one or more of the four alpha globin chains. In general, the alpha globin chains are structurally normal but are produced in reduced quantities. Most alpha thalassemia mutations result in gene deletion; non-deletional point mutations of an alpha globin gene are less common.

Individuals with two deleted copies of the alpha globin gene on the same chromosome are designated as **alpha zero thalassemia trait (designated --/)**. A single alpha globin gene deletion leads to a condition called **alpha plus thalassemia trait (designated -α/)**.

### Alpha Thalassemia syndromes

Genotype	Phenotype
αα/αα	Normal
-α/αα	Alpha plus thalassemia trait (heterozygote)
-α/-α	Alpha plus thalassemia trait (homozygote)
--/αα	Alpha zero thalassemia trait (heterozygote)
--/-α	Hemoglobin H disease
--/--	Hb Bart's Hydrops Fetalis (homozygous alpha thalassemia; alpha thalassemia major)

#### 1. Silent carrier (-α/αα)

Individuals who are heterozygous for an alpha **plus** thalassemia mutation (i.e. -α/αα) are generally silent carriers with normal hematological parameters and are asymptomatic.

#### 2. Alpha thalassemia trait (--/αα or -α/-α)

Alpha thalassemia trait occurs when there are two deleted alpha globin genes. These individuals are either heterozygous for alpha **zero** thalassemia (--/αα) or homozygous for alpha **plus** thalassemia (-α/-α). Both types of thalassemia trait are more or less identical in terms of the hematological findings and the individuals are usually asymptomatic.

Traditionally, alpha thalassemia is diagnosed in patients of the appropriate family origin (South East Asia, South Asia, Africa, Mediterranean/Middle East) who demonstrate:

- Thalassemic red cell indices, but particularly, a low MCV (< 80 fL) and/or MCH (< 27 pg)
- Hemoglobin H inclusions on supravital staining of red cells

#### Hemoglobin H inclusions

- Normal Hemoglobin A, the major hemoglobin species in adults, is comprised of two alpha globin chains and two beta globin chains (α<sub>2</sub>β<sub>2</sub>). In alpha thalassemia trait, especially alpha **zero** thalassemia, there is reduced production of alpha globin chains leading to an excess of beta globin chains. These beta globin chains form tetramers known as Hemoglobin H. Incubation of a peripheral blood specimen with a supravital dye leads to precipitation of the Hemoglobin H within the red cells which are visible by microscopy. A negative test for Hemoglobin H inclusions does not exclude a diagnosis of alpha thalassemia trait—the test is negative in up to 10% of patients with alpha **zero** thalassemia trait and frequently negative in alpha **plus** thalassemia trait.

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#### 3. Hemoglobin H Disease (--/-α)

If three of the four alpha globin genes are deleted, alpha chain production is reduced by about 75% which leads to a syndrome called Hemoglobin H disease. This is characterized by a hypochromic, microcytic, chronic hemolytic anemia and is sometimes accompanied by splenomegaly. These individuals have inherited a double gene deletion from one parent who has alpha **zero** thalassemia trait (--/αα) and a single gene deletion from the other parent who has alpha **plus** thalassemia trait (-α/-α or -α/αα).

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Because Hemoglobin H disease is usually a relatively mild disorder, identification of Hemoglobin H disease in a fetus is not generally considered to be an indication for prenatal testing.

#### 4. Hemoglobin Bart's Hydrops Fetalis; Homozygous alpha zero thalassemia (--/--)

This condition arises as a result of deletion of all four alpha globin genes so that the affected fetus is unable to produce Hemoglobin F or Hemoglobin A. Affected fetuses have profound anemia, cardiac failure with hydrops and die *in utero* or shortly after birth. There are also maternal morbidities associated with carrying a hydropic fetus. This condition arises from inheritance of alpha **zero** thalassemia from each parent. Genetic prenatal testing schemes are designed to detect couples at risk of producing a fetus with this syndrome.

#### Occurrence with beta thalassemia and hemoglobinopathies

It is important to recognize that alpha thalassemia is not uncommon in patients who have beta thalassemia trait and/or structural hemoglobinopathies (e.g. Hemoglobin E) since these conditions occur in individuals of similar ethnicities.

#### Why is it important to diagnose alpha thalassemia trait?

There are two main reasons:

1. To identify individuals who are carriers for alpha **zero** thalassemia who are at risk of having a fetus with Hemoglobin Bart's Hydrops Fetalis
2. To avoid a misdiagnosis of iron deficiency

As noted above, individuals with alpha **plus** thalassemia are not at risk of having a fetus with Hemoglobin Bart's Hydrops Fetalis but may have a baby with Hemoglobin H disease. Because Hemoglobin H disease is generally clinically mild, most genetic counselling and prenatal programs are not designed to prevent this condition; rather, these programs focus on Hemoglobin Bart's Hydrops Fetalis.

#### Molecular DNA analysis for alpha thalassemia

Molecular analysis of DNA using a polymerase chain reaction (PCR) has largely supplanted the Hemoglobin H inclusion test for the identification of alpha thalassemia trait since the latter is negative in a substantial number of affected patients (see above). The PCR test identifies most of the alpha **zero** thalassemia double gene deletions which are clinically significant; depending on the assay, some PCR tests will also identify non-deletional point mutations leading to alpha **plus** thalassemia. The PCR test used by BC Biomedical Laboratories will identify 5 alpha **zero** thalassemia mutations (--FIL; --THAI; --SEA; --MED; -20.5) and 16 alpha **plus** thalassemia mutations (-3.7; -4.2; and 14 other mutations).

#### Algorithm for testing for alpha thalassemia by PCR testing

The primary objective is to identify individuals or couples at risk of having a fetus with Hemoglobin Bart's Hydrops Fetalis, i.e. individuals or couples who are carriers for alpha **zero** (not **plus**) thalassemia. Alpha **zero** thalassemia is generally seen in individuals of South East Asian and Middle East/Eastern Mediterranean origin; it is extremely rare in individuals of South Asian (e.g. Indian, Pakistani) or African origin.

BC Biomedical Laboratories will be restricting PCR testing for **alpha** thalassemia to patients meeting the following criteria:

1. Patients < 45 years of age (patients of reproductive age or reproductive potential)
2. MCV < 80 fL and/or MCH < 27 pg
3. Patients with a family origin **other than** South Asia or Africa as determined from a patient Family Origin Questionnaire which the patient completes at the time of blood collection (in other words, patients of South Asian or African origin will **not** have a PCR test for alpha thalassemia since alpha **zero** thalassemia is extremely rare in these populations).

This algorithm conforms to the thalassemia/hemoglobinopathy screening programmes in use in the UK.<sup>1,2</sup>

Exceptions to this algorithm will be considered on a case-by-case basis by contacting a hematopathologist.

#### Testing for Beta thalassemia and structural hemoglobinopathies

The above algorithm applies only to testing for **alpha** thalassemia. BC Biomedical Laboratories will continue to test for **beta** thalassemia (Hemoglobin A2 quantitation) and structural hemoglobinopathies (capillary electrophoresis) in all patients regardless of their family origin.

#### Questions/concerns

Please address these to any member of the Hematopathology Group at 604-507-5000.

#### References

1. Sickle Cell and Thalassemia, Handbook for Laboratories. Second Ed. 2009, NHS Sickle Cell and Thalassemia Screening Programme (<http://sct.screening.nhs.uk>).
2. Ryan K, Bain B, Worthington D et al. Significant haemoglobinopathies: guidelines for screening and diagnosis. Brit J Haematol 2010;149:35-49.



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