Clinical Updates

TEST CHOICES FOR TAY SACHS DISEASE

When ordering Tay Sachs testing, it is critically important to specify the particular Tay Sachs test desired. Enzyme assays for Hexosaminidase A and Total, and genetic tests for HEXA gene mutations are now available.

If the patient is either pregnant or taking birth control pills, the enzyme test must be performed on whole blood (leukocytes). For non-pregnant patients who are not on birth control pills, serum is used for testing.

Genetic testing is performed on either whole blood or amniocytes (cultured cells from amniotic fluid). Test options are listed below.

**ENZYME ASSAYS**

0092471: Hexosaminidase A & Total, on Leukocytes
- **Collect:** One 7 mL yellow (ACD Solution A or B) whole blood (Min: 5mL) from pregnant patient or patient on birth control pills.
- **Stability:** Refrigerated 2-8°C up to 48 hours. Ambient: Unacceptable; Frozen: Unacceptable.
- **Note:** This is a CRITICAL TIME specimen sent to a reference laboratory & must be received in the laboratory in time to meet shipping deadlines. Collect samples Monday through Thursday.

0095269: Hexosaminidase A & Total, on Serum
- **Collect:** One 10 mL plain red top tube from non-pregnant patient. Separate serum from cells ASAP.
- **Stability:** After separation from cells: Refrigerated:1 week; Frozen:1 week; Ambient: unacceptable.

**GENETIC TESTING**

0051428: Tay-Sachs Disease, HEXA Gene Mutations
- **Collect:** One 3 mL lavender (EDTA) or pink (K$_2$EDTA), 3 mL whole blood (Min:1 mL)
- **Stability:** Ambient: 3 days; Refrigerated: 1 week; Frozen: Unacceptable

0051429: Tay-Sachs Disease, HEXA Gene Mutations, Fetal
- **Collect:** Amniotic fluid. Culture amniocytes.
- **Transport cultured amniocytes:** Two T-25 flasks at 80% confluence. Fill flasks with culture media to ship at 20-25°C. This is a CRITICAL TIME specimen sent to a reference laboratory. Samples must be received in the laboratory in time to meet shipping deadlines. Send samples to lab Monday through Thursday.
- **Remarks:** Maternal specimen (EDTA or ACD) is recommended for proper test interpretation.
- **Order Maternal Cell (MCC MAT) (0050608).**
- **Stability:** Fetal: Ambient: 48 hours; Refrigerated: 48 hours; Frozen: Unacceptable
  Maternal: Ambient: 24 hours; Refrigerated: 5 days; Frozen: Unacceptable
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If you wish to obtain additional information, a genetic counselor at our reference laboratory ARUP is available to assist you. Please contact Chris Miller in the Molecular Genetics Department, at 800-242-2787, x2946. Chris's hours of work are from 8:30-5:00 MST, M-Fri.
Tay-Sachs Disease

Tay-Sachs disease is a rare inherited disorder that causes progressive destruction of nerve cells in the brain and spinal cord (the central nervous system). The most common form of Tay-Sachs disease begins in infancy. Infants with this disorder typically appear normal until the age of 3 to 6 months, when development slows and muscles used for movement weaken. Affected infants lose motor skills such as turning over, sitting, and crawling. They also develop an exaggerated startle reaction to loud noises. As the disease progresses, children with Tay-Sachs disease experience seizures, vision and hearing loss, mental retardation, and paralysis. An eye abnormality called a cherry-red spot, which can be identified with an eye examination, is characteristic of this disorder. Children with this severe infantile form of Tay-Sachs disease usually survive only into early childhood.

In accordance with guidelines from the American College of Obstetrics and Gynecology and the American College of Medical Genetics, carrier testing for Tay-Sachs disease should be offered to individuals whose ancestry is fully or partially Jewish. As well, other groups with a high frequency of Tay-Sachs carriers should be offered testing, including Pennsylvania Dutch, Cajuns of Southern Louisiana, and French Canadians of Eastern Quebec.

DNA mutation analysis is less prone to error and is the preferred method of prenatal diagnosis when one of the common mutations is identified. Enzyme analysis for prenatal diagnosis should be reserved for cases where the mutation is unidentified.

Limitations to the enzyme assay:
1. There is an indeterminate range where carriers and non-carriers may overlap.
2. The serum assay is unreliable in pregnant women and in women taking oral contraceptives.
3. Carriers of pseudodeficiency alleles cannot be distinguished from carriers of disease-causing mutations.

Limitations to DNA testing:
DNA diagnosis for Tay-Sachs disease and carrier status involves testing for common mutations only. Clinical labs do not search for all (100) mutations that have been reported to date.

Attached is an excerpt from Screening and Counseling Families at Risk for Metabolic Disease by V. Reid Sutton, MD describing both methods of testing.

Excerpt from Screening and Counseling Families at Risk for Metabolic Disease by V. Reid Sutton, MD
In published studies of Ashkenazi Jewish testing programs, it is estimated that the false positive rate of serum enzyme assay is 1.1% (excluding pseudodeficiency cases), giving a specificity of 98.9%. Of those tested by enzyme assay, 10% were in the inconclusive range and further testing (either by enzyme assay on leukocyte/platelet or by DNA mutation analysis) was required. Of those in the inconclusive range 2% were found to be carriers by DNA mutation analysis; 98% had no pathogenic mutation detected. Because of these limitations, abnormal or inconclusive results of enzyme assay should be clarified with enzyme assay on leukocytes and/or DNA mutation analysis. Women who are pregnant or taking oral contraceptives may have elevated serum Hex A enzyme levels, that will
result in an inability to detect that they are carriers (false negative results). If the individual to be tested is taking oral contraceptives, or is pregnant, the enzyme assay should be done on leukocytes, not on serum. If pregnant, the DNA mutation analysis should be sent at the same time as the enzyme assay, since expeditious identification of carrier status is important. The use of an artificial substrate for assay of Hex A and Hex B enzyme activity confers simplicity and low cost. However, the artificial substrate causes false positive results in 2% of Ashkenazi Jews and 35% of non-Jews. This is because of two common variations (polymorphisms) in the HEXA gene (R247W and R249W) that impair Hex A activity against the artificial substrate used in the enzyme assay but do not impair the ability of Hex A to hydrolyze the natural substrate, GM2 ganglioside. Thus, individuals whose enzyme assay indicates carrier status should have DNA mutation analysis for the two common pseudodeficiency alleles (R247W and R249W). Rarely, individuals will carry both a pseudodeficiency allele and a disease causing mutation. The enzyme assay results will be in the range of individuals affected with Tay-Sachs disease, though the individual will not have a Tay-Sachs disease phenotype. DNA mutation analysis for common mutations and pseudodeficiency alleles can clarify the enzyme results in these cases. All individuals who are in the carrier or indeterminate range on enzyme analysis should have DNA testing to verify the results and in anticipation of prenatal testing.

(For genetic testing) DNA is extracted from blood or cultured amniocytes/chorionic villus cells and the DNA is amplified by polymerase chain reaction (PCR). The entire HEXA gene is not amplified; rather the particular areas of the HEXA gene where common mutations occur are amplified and then used in testing. Most labs use some reliable and sensitive methodology, such as allele specific oligonucleotide hybridization (ASO, aka dot-blot) testing. These amplified DNA fragments are hybridized with oligonucleotide (short sequences of single stranded DNA synthesized to specifically match either the normal or mutant DNA sequence). The process of hybridization is where the labeled single stranded oligonucleotide joins with the complementary PCR product to form a double stranded piece of DNA. A radioactive detection method is typically used. Although more rapid methods may soon come into wide use, it is unlikely that there will be expansion of the number of mutations tested. It is important to know which mutations the laboratory is testing for. Some labs test for only the three common disease-causing Ashkenazi Jewish mutations. These labs, while appropriate for testing Ashkenazim, are not appropriate for testing non-Jews. The clinician ordering the test should check with the lab to be sure that they test for the common mutations seen in a particular population. For example, if a non-Jew is being tested, the laboratory selected should test for the 1073 + 1G→A mutation, which accounts for 15% of mutations in the non-Jewish population. Sensitivity of testing for those of Ashkenazi Jewish descent is >98% when testing for the three most common alleles (1277insTATC, 1421 + 1G→C, and G269S). The carrier frequency in Ashkenazi Jews is 1/30; the DNA test false negative rate is < 2% (2/100). Therefore, in Tay-Sachs carrier testing of Ashkenazim, one out of every 1500 (1/30 X 2/100) carriers will be missed by DNA testing. Put the other way around, 1499/1500 Ashkenazi carriers will be correctly identified. Thus, 99.9% of Ashkenazi Jewish carriers of Tay-Sachs disease are identified by DNA mutation analysis for the three most common alleles. Interpreting results testing of both Ashkenazi Jewish individuals and non-Jews for Tay-Sachs carrier status may utilize enzyme analysis, DNA diagnostics, or both studies in combination. Two percent of Ashkenazi Jews and 35% of non-Jews who have enzyme analysis results in the carrier range will carry pseudodeficiency alleles and not disease causing mutations. As well, DNA testing can confirm carrier status, and identification of the disease causing mutation can permit accurate
prenatal diagnosis. Thus, all individuals found to be carriers or in the indeterminate range by enzyme assay should have DNA mutation analysis. Those who have enzyme activity in the carrier range and have a pseudodeficiency allele (R247W or R249W) are not carriers for Tay-Sachs disease. Therefore, prenatal diagnosis is not indicated. Those found to have a disease causing mutation (1278insTATC, 1421 + 1G→C, G269S, 1073 + 1G→A) are carriers for Tay-Sachs disease. They should be counseled that they are carriers and offered prenatal testing by DNA analysis of cultured amniocytes/chorionic villus cells if the partner is also a carrier of a known mutation. Those who have carrier range serum enzyme results and no disease causing or pseudodeficiency mutation detected should have a leukocyte enzyme assay. If this is also indicative of carrier status, the individual should be counseled that he/she is a carrier and offered prenatal testing (by enzyme assay of cultured amniocytes/chorionic villus cells) if the other partner is also a carrier.

Summary
Because Tay-Sachs is an autosomal recessive disease, there do not have to be prior affected family members for an individual to be a carrier or for a couple to be at risk. Therefore, individuals should not be reassured by the absence of genetic disease in their family. Counseling should be provided by an obstetrician, genetic counselor, or geneticist who has an understanding of methods of testing, can interpret test results, and convey the options for couples at-risk. Individuals offered testing should understand the following points:

- Individuals of Jewish ancestry have a 1/30 chance of being a carrier; if both partners are of Jewish ancestry their risk of both being carriers is 1/900 (1/30 X 1/30), even if there are no prior affected individuals in either family.
- Testing using DNA analysis offers > 99% certainty of carrier status in Ashkenazi Jews.
- If both prospective parents are carriers, there is a 25% risk of having an affected child with each pregnancy.

Carrier testing should be done prior to becoming pregnant, or as soon as an expectant couple comes to medical attention. This is to ensure that individuals can give full consideration to their options for testing and intervention. Testing of pregnant women using enzyme assay is also more complicated because pregnancy can alter serum levels of Hex A and therefore enzyme analysis of pregnant women must be done on leukocytes.
If only one member of a couple is of Ashkenazi Jewish descent, that person should be tested first. This is because the enzyme assay and DNA test are more accurate in Ashkenazi Jews. They have a much lower false positive carrier status secondary to pseudodeficiency alleles (2% in Ashkenazim versus 35% in non-Jews).

In addition to all the mutations listed below the **Tay-Sachs Disease, HEXA Gene Mutations** and **Tay-Sachs Disease, HEXA Gene Mutations, Fetal** panels tests for the mutation (Del7.6kb).
**Gene Mutations Identified and Ethnic Percentages**

**Molecular Genetic Testing Used in Hexosaminidase A Deficiency**

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Mutations Detected</th>
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<th>Heterozygotes</th>
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</tr>
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<td></td>
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</tr>
<tr>
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<td>Pseudo-deficiency</td>
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<tr>
<td>All of the above</td>
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<td>98%</td>
<td>46%</td>
</tr>
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*From Kaback et al 1993*