Pelizaeus-Merzbacher Disease (PMD)/Spastic Paraplegia 2 (SPG2): *PLP1*

Autosomal Dominant Leukodystrophy, adult onset (ADLD): *LMNB1*

Pelizaeus-Merzbacher-Like Disease (PMLD): *GJC2*

Hypomyelination and Congenital Cataract (HCC): *FAM126A*

Thank you for contacting us about our tests. If you have questions, or if you did not receive the requisition form and Specimen instructions form, please call 302-651-6775 or 302-651-6829 for assistance.

CLIA#: 08D0706140; expires: 08/29/2013.

Turnaround time is four to six weeks. Contact the lab if quicker turnaround is necessary.

1. **Pelizaeus-Merzbacher Disease** (PMD; MIM: 312080) and **Spastic Paraplegia 2** (SPG2; MIM: 312920) are X-linked recessive diseases caused by duplications or mutations of the proteolipid protein 1 gene (*PLP1*; MIM: 300401; GenBank Accession No. NM_000533). Information about the diseases and indications for testing can be found in the GeneReview entitled *PLP1 Related Disorders* at this website: [http://www.genetests.org](http://www.genetests.org). Search on PLP1.

2. **Pelizaeus-Merzbacher-Like Disease** (PMLD; MIM: 608804) is an autosomal recessive disease caused by mutations of the gap junction protein, gamma 2 gene (*GJC2*; MIM: 608803; GenBank Accession No. NM_020435). Symptoms are similar to those of PMD (Uhlenberg et al., American Journal of Human Genetics 75:251-260, 2004). There is a report in the literature that autosomal dominant missense mutations in GJC2 cause lymphedema (Ferrell et al., American Journal of Human Genetics 86:943-948, 2010).

3. **Autosomal Dominant Leukodystrophy, adult-onset** (ADLD; MIM: 169500) is caused by duplication of the nuclear lamina protein lamin B1 (*LMNB1*; MIM: 150340; GenBank Accession No. NM_005573). One of the first articles described familial disorder similar to Pelizaeus-Merzbacher Disease except later onset and autosomal dominant inheritance. The identification of the genomic duplication was reported by Padiath et al., Nature Genetics 38(10):1114-23, 2006.

4. **Hypomyelination and Congenital Cataract** (HCC; MIM: 610532) is an autosomal recessive disorder caused by mutations of the *FAM126A* (also know as DRCTNNB1A or Hyccin; MIM: 610531; GenBank Accession NG_008392.1). The identification and deficiency of this novel membrane protein were characterized by Zara et al., Nature Genetics 38(10):1111-3, 2006.

- We do not bill third party payers (insurance companies) for samples received from external sources. The person or institution sending the sample is responsible for full payment of the invoices within 30 days of receipt of the invoice.
Description of tests

Duplication screening – PMD (PLP1) and ADLD (LMNB1)

**Proband, Family Member or Carrier:** The number of copies of the PLP1 and LMNB1 genes is determined by multiplex PCR amplification with segments of reference genes on the autosomal, X and Y chromosomes. The amplified regions for PLP1 are exons 1, 5 and 7; and for LMNB1 are exon 5 and exon 11 (coding region into the 3’ UTR). Primers used in the PCR amplification are labeled with a fluorescent dye, PCR products are separated by electrophoresis, and the quantity of each PCR product is determined by measuring intensity of fluorescence. The number of copies of a gene is calculated based on the normalized ratio of the gene to each reference gene in the multiplex reaction for the patient, normal controls and duplicated controls. The tests for males and females are separate tests.

Mutation detection – PMD/SPG2 (PLP1)

**Male proband:** Sequence analysis is performed in one direction on the seven coding regions of the PLP1 gene and intron-exon junctions to identify a mutation. PCR-based automated fluorescent sequencing is used. Sequence analysis is >99% accurate, but rare variation in the DNA of individuals may sometimes cause uncertainty in interpretation of the results. A negative result will exclude the presence of a mutation (or mutations) in the regions of the PLP1 gene tested. However, a negative result does not exclude the possibility that other mutations are present in other regions of the PLP1 gene or in other genes.

**Female proband:** Sequence analysis is performed in both directions on the seven coding regions of the PLP1 gene and intron-exon junctions to identify a mutation. PCR-based automated fluorescent sequencing is used. Sequence analysis is >99% accurate, but rare variation in the DNA of individuals may sometimes cause uncertainty in interpretation of the results. A negative result will exclude the presence of a mutation (or mutations) in the regions of the PLP1 gene tested. However, a negative result does not exclude the possibility that mutations are present in other regions of the PLP1 gene or in other genes.

**Male family member:** Sequence analysis is performed in two directions on one exon of the PLP1 gene and its intron-exon junctions to identify a mutation found to be present in a family member. PCR-based automated fluorescent sequencing is used. Sequence analysis is >99% accurate, but rare variation in the DNA of individuals may sometimes cause uncertainty in interpretation of the results. A negative result will exclude the presence of the family mutation in the sample. However, a negative result does not exclude the possibility that mutations are present in other regions of the PLP1 gene or in other genes.

**Female carrier, mutation detection:** Sequence analysis is performed in both directions on one exon of the PLP1 gene and its intron-exon junctions to identify a mutation found to be present in an affected family member. PCR-based automated fluorescent sequencing is used. Sequence analysis is >99% accurate, but rare variation in the DNA of individuals may sometimes cause uncertainty in interpretation of the results. A negative result will exclude the presence of the family mutation in the sample. However, a negative result does not exclude the possibility that mutations are present in other regions of the PLP1 gene or in other genes.

Mutation detection – PMLD (GJC2/connexin 46.6)

**Proband, mutation detection:** Sequence analysis is performed in both directions on the 5’ untranslated region, one coding region of the GJC2 gene, and the intron-exon junctions to identify a mutation. PCR-based automated fluorescent sequencing is used. Sequence analysis is >99% accurate, but rare variation in the DNA of individuals may sometimes cause uncertainty in interpretation of the results. Fragment analysis will also be performed to detect a whole or partial gene deletion. A negative result will exclude the presence of a mutation (or mutations) in the regions of the GJC2 gene tested. However, a negative result does not exclude the possibility that mutations are present in other regions of the GJC2 gene or in other genes.
Family Member, mutation detection: Sequence analysis is performed in both directions on the regions of the GJC2 gene found to contain mutations in a proband. PCR-based automated fluorescent sequencing is used. Sequence analysis is >99% accurate, but rare variation in the DNA of individuals may sometimes cause uncertainty in interpretation of the results. A negative result will exclude the presence of the family mutation (or mutations). However, a negative result does not exclude the possibility that mutations are present in other regions of the GJC2 gene or in other genes.

Mutation detection – HCC (FAM126A)

Proband, mutation detection: Sequence analysis is performed in both directions on the 5' untranslated region, 11 coding regions of the FAM126A gene, and the intron-exon junctions to identify a mutation. PCR-based automated fluorescent sequencing is used. Sequence analysis is >99% accurate, but rare variation in the DNA of individuals may sometimes cause uncertainty in interpretation of the results. A negative result will exclude the presence of a mutation (or mutations) in the regions of the FAM126A gene tested. However, a negative result does not exclude the possibility that mutations are present in other regions of the FAM126A gene or in other genes.

Family Member, mutation detection: Sequence analysis is performed in both directions on the regions of the FAM126A gene found to contain mutations in a proband. PCR-based automated fluorescent sequencing is used. Sequence analysis is >99% accurate, but rare variation in the DNA of individuals may sometimes cause uncertainty in interpretation of the results. A negative result will exclude the presence of the family mutation (or mutations). However, a negative result does not exclude the possibility that mutations are present in other regions of the FAM126A gene or in other genes.

Possible diagnostic errors include sample mix-up and genotyping errors resulting from trace DNA contamination of PCR reactions. These tests were developed and their performance characteristics determined by our laboratory. They have not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. These tests are used for clinical purposes.

Grace M. Hobson, PhD
Director, Molecular Diagnostics for PMD
(302) 651-6829
PROCEDURE FOR SAMPLE SUBMISSION

We normally process two types of samples:

1. DNA SAMPLES

2. BLOOD SAMPLES

* Draw two 3 cc vacutainer tubes containing liquid EDTA (purple top tubes). If the patient is a small child, it is possible to use just 3 cc.

** Ship tubes with cold pack in durable container (can be shipped at ambient temperature if no cold pack is available).

*** Do not freeze the samples. Samples drawn on a Friday should be kept in the refrigerator for shipping on Monday. Please be aware that we are closed on holidays and samples should be shipped accordingly.

SHIPPING:

* Ship samples by overnight delivery to:

  Susan Kirwin  
  Nemours/ A.I. duPont Hospital for Children  
  Molecular Diagnostics Lab, Room 256  
  1600 Rockland Road  
  Wilmington, DE 19803  
  302-651-6775

*** Please fill in the submission form: we need to know which physician should receive the report and we need to know whom to bill.
Facsimile Verification Form

Name of Facility receiving Fax: __________________________________________________________

Name of Physician/Lab receiving Fax: _____________________________________________________

Street Address: _______________________________________________________________________

City_________________________ State: ______

Fax Number: ____________________________
(to which lab results and/or patient information may be sent)

Phone Number: __________________________

By signing this Facsimile Verification Form, I validate the accuracy of the above information and assume responsibility for assuring that the Fax machine is in a location which will maintain confidentiality of all reports transmitted by the Molecular Diagnostics Laboratory of the Nemours/Alfred I. duPont Hospital for Children, to the above fax number.

Authorized Contact Person: _____________________________________________________________

Signature: ___________________________ Date: __________________

Title: __________________________

In our continuing efforts to maintain patient confidentiality, the Molecular Diagnostics Laboratory of the Nemours/Alfred I. duPont Hospital for Children requests you to verify the fax number only once from your medical practice or institution and to assure that all faxes regarding patient information are received in a secure location in accordance with HIPAA regulations.

Please complete this Facsimile Verification Form and fax back to 302.651.6795.
If you have any questions regarding this form please contact Susan Kirwin, Assistant Director of the Molecular Diagnostics Laboratory, at 302.651.6777.

This is a confidential document that is being sent from a fax machine in a secure location.
This fax is covered by the Electronic Communications Privacy Act 18 U.S.C. 2510-521. The information contained in this fax is considered privileged, is otherwise confidential and is intended only for the use of the individual or entity named above. Dissemination, distribution, or copying of this communication is strictly prohibited. If you have received this communication in error please notify us immediately by telephone, and return the original message to us at the above address via the U.S. Postal Service.
## Credit Card Billing Information

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If the patient prefers to pay via credit card - Please have them complete this form and include the paperwork with the shipment of the sample.

Billing questions can be addressed to: Denise Axsmith
Senior Budget/Financial Analyst
Nemours/A.I. duPont Hospital for Children
daxsmith@nemours.org
Phone: 302.651.6802
Fax: 302.651.6881