



Lawrence M. Weiss, MD  
Laboratory Director, CLIA #05D0665695  
Juan-Sebastian Saldivar, MD, FACMG  
Director of Molecular Diagnostics

**Molecular Diagnostic Laboratory**  
1500 East Duarte Road  
Northwest Building, Second Floor, Room 2236  
Duarte, CA 91010-3000  
Phone 888-826-4362 Fax 626-301-8142  
mdl@coh.org <http://mdl.cityofhope.org>

## Assay Summary

### Neuroigin 3 and 4 Gene Mutation Analysis

#### PDD, Autism and Asperger's Assay

##### *Synopsis*

Autism and Asperger syndrome fall within a group of disorders classified as pervasive developmental disorders, or PDD. Some argue that Autism and Asperger represent two severities along a single disease continuum. The prevalence of these diseases is estimated to be around 40 per 10,000 for autism and 67 per 10,000 for all PDD's, according to a report by the CDC in 2000. The principle characteristics of these diseases are impairments in social interaction, stereotypic/repetitive behaviors, and impairments in communication (not so for Asperger's). About 2/3's of patients with Autism are mentally retarded. About 25% of children with Autism will also have other dysmorphic features such as microcephaly and structural brain abnormalities. No single gene has surfaced to explain the majority of these conditions despite heritability estimates of more than 90%, suggesting multifactorial inheritance. Work done by Jamain et al., along with supportive findings in our lab, establish a role for neuroigin in the pathogenesis of Autism and Asperger's.

The neuroigin gene family (NLGN1, 2, 3, 4, and 4Y) codes for cell adhesion molecules situated in the post-synaptic membranes. They interact with neuexins for the maintenance of normal synaptic functions. A triad of studies initially reported the association between neuroigin and autism, Asperger syndrome, and mental retardation. Jamain et al. reported a NLGN3 missense mutation in a pair of Swedish brothers, one with autism, and the other with Asperger syndrome. They also reported a NLGN4 frameshift mutation in another pair of affected brothers, one with autism and the other with Asperger syndrome<sup>1</sup>. Laumonnier et al. reported a NLGN4 frameshift mutation in a French family in which all affected family members had autism or non-autistic mental retardation<sup>2</sup>. They also demonstrated co-segregation with disease in six affected and three unaffected individuals within the family. Additionally, they presented the mother of an affected individual, a 34-year-old obligate carrier, who had evidence of mental retardation. Yan et al. reported four NLGN4 missense changes in U.S. Midwest and Portuguese Caucasian families with autism at a frequency of 3% in each population, and no structural changes were found in controls (P=0.009)<sup>3</sup>.

##### *Indications for testing*

Individuals with a clear diagnosis of Autism or Asperger's, with or without a family history of the disease. To help a diagnosis in suspected Autism or Asperger's. Once a specific mutation is known in a family, presymptomatic testing for appropriate family members can be performed.

##### *Methodology*

All coding exons and associated intron junctions of the NLGN3 and NLGN4 genes are analyzed by direct DNA sequence analysis using an automated fluorescent sequencer. When a mutation is detected, confirmation is carried out by sequencing in the opposite direction, in an second independent PCR amplification. If no mutation is found, sequence analysis is performed in both directions. Testing of at-risk family members can be offered by DNA sequence analysis of only the region of the gene with the previously identified mutation.

### ***Performance/limitations***

Based on the reported literature, and our clinical experience of over 250 cases, we expect a detection rate of about 2-4% in individuals with autism or Asperger syndrome, +/- other developmental delay features. For mutation analysis, the method will not detect mutations located in regions of the genes that are not analyzed (non-coding exon sequences, intron sequences other than the splice junctions, and upstream and downstream sequences). The method also will not detect gross genetic alterations including most large deletions, duplications, and inversions. Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance. Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

### ***Specimen Requirements***

(a) Blood samples: 2 tubes (6 ccs each) in ACD (yellow top) or EDTA (lavender top) tubes.  
Keep ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

**Note:**

- i) for infants, a minimum of 6 ccs is sufficient
- ii) we accept DNA; at least 10 micrograms

(b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W/Th.  
A blood sample from the mother is also required (6 ccs in ACD, yellow top) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis on X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

### ***Test Request Form (TRF)***

A completed MDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.

<b><i>Order Codes</i></b>	<b><i>CPT Codes</i></b>	<b><i>TAT</i></b>
NLGN3-SEQ (NLGN3 gene, full gene sequencing)	83890, 83898(x8), 83894, 83904(x8), 83912	6 wks
NLGN3-CAS (NLGN3 gene, targeted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	4 wks
NLGN3-PD (NLGN3 gene, prenatal, targeted mutation analysis)	83890, 83898, 83894, 83904, 83912	2 wks
NLGN4-SEQ (NLGN4 gene, full gene sequencing)	83890, 83898(x9), 83894, 83904(x9), 83912	6 wks
NLGN4-CAS (NLGN4 gene, targeted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	4 wks
NLGN4-PD (NLGN4 gene, prenatal, targeted mutation analysis)	83890, 83898, 83894, 83904, 83912	2 wks

### ***References***

1. Jamain S. et al., (2003) *Nat. Genet.* **34(1)**:27-29
2. Laumonnier et al., (2004) *Am. J. Hum. Genet.* **74(3)**:552-557
3. Yan et al., (2005) *Mol. Psy.* **10(4)**:329-332

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc.