Condition Description

In the United States, approximately 1 in 1000 children are diagnosed with prelingual hearing loss (HL) or deafness. Approximately half of prelingual hearing loss or deafness is attributed to environmental exposures and the remaining half to genetic causes. Approximately 30% of hereditary hearing loss is estimated to be syndromic (associated with other birth defects) while the remaining 70% is non-syndromic (isolated and not associated with other findings). Non-syndromic deafness is mainly due to recessive genes (75-80%) and over 20 such genes have been identified, but non-syndromic deafness may also be inherited in autosomal dominant, X-linked, or mitochondrial patterns.

Molecular testing can aid in rapid diagnosis of hearing loss. Early diagnosis of hearing defects can provide diagnostic information, facilitate timely intervention, and assist with genetic counseling.

Connexins are transmembrane proteins that form channels that allow rapid transport of small molecules between cells; the proteins Connexin 26 and 30 interact to form a channel that functions in the inner ear. The GJB2 gene encodes the Connexin 26 protein and is involved in 50% of autosomal recessive hearing loss. The GJB6 gene is located near GJB2, and encodes the protein Connexin 30. Patients with non-syndromic hearing loss have been found to have two mutations in connexin 26, two mutations in connexin 30, or compound heterozygosity for one mutation in connexin 26 and another in connexin 30 [1,2].

In the presence of specific mitochondrial DNA (mtDNA) mutations, moderate to severe hearing loss can result from exposure to aminoglycoside antibiotics such as gentamycin, tobramycin, amikacin, kanamycin, or streptomycin [3]. Pathogenic variants in the mitochondrial MTRNR1, MTCO1, and MTTS1 genes have been associated with aminoglycoside ototoxicity in an estimated 2% of deaf individuals in the US [4-5]. The prevalence is higher, 15-30%, among deaf persons with a history of aminoglycoside exposure [6]. One of the most common mitochondrial pathogenic variants is the m.1555A>G substitution in the MTRNR1 gene which can be found in 0.6-2.5% of Caucasian, 3-5% of Asian and as high as 17% of the Spanish population with non-syndromic hearing loss [7].

The mitochondrial variants m.7,445A>G/m.7,443A>G/m.7,444G>A in the tRNA serine gene (MTCO1 and MTTS1) have been found in patients with maternally inherited sensorineural hearing loss, but they are less likely to cause aminoglycoside hypersensitivity. Of individuals with mitochondrial non-syndromic hearing loss, 14% have pathogenic variants m.7443A>G, m.7444G>A, or m.7455A>G. Most mitochondrial DNA mutations causing non-syndromic hearing loss are maternally inherited. However, heteroplasmic states (uneven distribution of mitochondrial DNA during cell division) and variable penetrance may be related to the level of mutant mitochondria present, and is not quantitated by this assay.

The comprehensive hearing loss panel provides a comprehensive approach to test for the underlying genetic etiology of non-syndromic hearing loss.

The panel includes complete sequencing of the genes for connexin 26 and 30, testing for the common 342kb deletion in connexin 30, and testing for four specific mitochondrial mutations associated with hearing loss.

Please click here for the GeneReviews summary on deafness and hereditary hearing loss.

References:


Genes

- **GJB2, GJB6**

Indications

This test is indicated for:

- Individuals with clinical findings consistent with non-syndromic hearing loss.
- Carrier testing in individuals with a family history of non-syndromic hearing loss.

Methodology

PCR amplification of the exons and flanking regions contained in the GJB2 and GJB6 genes are performed on the patient’s genomic DNA. Direct
sequencing of amplification products is performed in both forward and reverse directions using automated fluorescence dideoxy sequencing methods. The patient’s gene sequences are then compared to a normal reference sequence. Sequence variations are classified as mutations, benign variants unrelated to disease, or variations of unknown clinical significance. Variants of unknown clinical significance may require further studies of the patient and/or family members. This assay does not interrogate the promoter region, deep intronic regions, or other regulatory elements, and does not detect large deletions. The GJB6 gene 342kb deletion is detected by allele-specific amplification.

The m.1555A>G and m.7,445A>G/m.7,443A>G/m.7,444G>A mitochondrial variants are assayed by PCR and Sanger sequencing.

**Detection**

**GJB2 Sequencing:**
Detects over 98% of sequence variants in the coding region and splice junctions.

**GJB6 Sequencing:**
It is possible that some patients with a typical presentation may not carry a mutation detected by this analysis. This analysis may detect novel variants of unclear effect, which may require further studies. Mutations in the promoter region, some mutations in the introns, and other regulatory elements cannot be detected by sequence analysis. Large deletion such as the most common 342 kb deletion, and insertions will not be detected by sequence analysis.

**GJB6 Deletion:**
Will detect nearly all 342kb common deletion alleles in Connexin 30. Other deletion mutations reported in GJB6, including the 232kb (reported in Spain), 309kb (reported in UK), 140kb, and 150kb deletions will be detected by the separate GJB6 deletion/duplication array. This panel is estimated to detect about 35% cases of all mitochondrial non-syndromic hearing loss.

**Reference Range**
Qualitative assay.

**Specimen Requirements**
Submit only 1 of the following specimen types

**Type: DNA, Isolated**
Specimen Requirements:
Microtainer
8µg
Isolation using the Perkin Elmer™Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

Specimen Collection and Shipping:
Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

**Type: Saliva**
Specimen Requirements:
Oragene™ Saliva Collection Kit
Orangene™ Saliva Collection Kit used according to manufacturer instructions. Please contact EGL for a Saliva Collection Kit for patients that cannot provide a blood sample.

Specimen Collection and Shipping:
Please do not refrigerate or freeze saliva sample. Please store and ship at room temperature.

**Type: Whole Blood (EDTA)**
Specimen Requirements:
EDTA (Purple Top)
Infants and Young Children (2 years of age to 10 years old): 3-5 ml
Older Children & Adults: 5-10 ml
Autopsy: 2-3 ml unclotted cord or cardiac blood

Specimen Collection and Shipping:
Ship sample at room temperature for receipt at EGL within 72 hours of collection. Do not freeze.

**Related Tests**

- Hearing Loss: Connexin 26 (GJB2) Sequencing (OZ) along with Hearing Loss: Connexin 30 Sequencing/Deletion (SX) is available when mitochondrial etiologies have been ruled out
- Hearing Loss: Mitochondrial-Related Mutation Panel (OJ) for patients with a history of aminoglycoside sensitivity.
- For patients with mutations not identified by full gene sequencing, Hearing Loss: Connexin 26 and 30 Deletion/Duplication (PI) is available. Refer to the test requisition or contact the laboratory for more information.
- Known Mutation Testing (KM) is available to family members if mutations are identified by sequencing.
- Prenatal testing is available to couples who are confirmed carriers of mutations. Please contact the laboratory genetic counselor to discuss appropriate testing prior to collecting a prenatal specimen.

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