Steroid-Resistant Nephrotic Syndrome Panel: Sequencing and CNV Analysis

Test Code: MM620
Turnaround time: 6 weeks
CPT Codes: 81406 x1, 81404 x1, 81405 x1, 81407 x1

Condition Description
Nephrotic syndrome is a collection of signs and symptoms that occur when glomeruli (the filtering units of the kidneys) leak protein into the urine. Clinical findings include increased proteinuria, edema (swelling), hyperlipidemia, and hypoalbuminemia. Nephrotic syndrome is one of the most commonly diagnosed kidney diseases in childhood and can occur in adulthood. Every year, 2-4 out of every 100,000 children and 3 out of every 100,000 adults are diagnosed with primary nephrotic syndrome in North America.

Nephrotic syndrome can present as an isolated finding or in association with nephritis syndrome or another syndrome. Several diseases may cause nephrotic syndrome and prognosis varies depending on causation. Primary causes include minimal-change nephropathy, focal glomerulosclerosis, membranous nephropathy, and hereditary nephropathies. Secondary causes include diabetes mellitus, lupus erythematosus, viral infections, and preeclampsia. Progressive forms of nephrotic syndrome can lead to chronic kidney disease and/or end-stage renal disease.

Genetic disorders account for the majority of cases of nephrotic syndrome that begin within the first year of life and in childhood. Treatment may be available depending on causation of the syndrome, and/or underlying genetic defect. Identification of mutations may modify genetic counseling and recurrence risks after renal transplantation. Phenotypic variation has been demonstrated in patients with the same mutations and/or mutations within the same gene. When a hereditary disorder is suspected, age of onset, presence of extrarenal abnormalities, and type of renal histologic lesions should be considered to determine the appropriate gene(s) to test.

References:
2. Medscape (Pediatric Nephrotic Syndrome and Nephrotic Syndrome)

Genes
ACTN4, ARHGAP24, ARHGDA1, CD2AP, CFH, COL4A3, COL4A4, COQ2, COQ6, COQ8B, CRB2, CUBN, DGKE, EMP2, INF2, ITGA3, ITGB4, KANK1, KANK2, LAMB2, LMX1B, MEFV, MYO1E, NEIL1, NPHS1, NPHS2, NUP107, NXF5, OCRL, PAX2, PDSS2, PLCE1, PMM2, PTPRO, SCARB2, SMARCAL1, TRPC6, TTC21B, WDR73, WT1

Indications
The test is indicated for:
- Individuals with a clinical diagnosis of nephrotic syndrome.

Methodology
Next Generation Sequencing: In-solution hybridization of all coding exons is performed on the patient's genomic DNA. Although some deep intronic regions may also be analyzed, this assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications. Direct sequencing of the captured regions is performed using next generation sequencing. The patient's gene sequences are then compared to a standard reference sequence. Potentially causative variants and areas of low coverage are Sanger-sequenced. Sequence variations are classified as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance. Variants of unknown significance may require further studies of the patient and/or family members.

Due to pseudogenes, exons 8-10 and exons 20-22 of the CFH gene and exons 41-50 and exons 63-67 of the CUBN gene cannot be analyzed at this time.

Copy Number Analysis: Comparative analysis of the NGS read depth (coverage) of the targeted regions of genes on this panel was performed to detect copy number variants (CNV). The accuracy of the detected variants is highly dependent on the size of the event, the sequence context and the coverage obtained for the targeted region. Due to these variables and limitations a minimum validated CNV size cannot be determined; however, single exon deletions and duplications are expected to be below the detection limit of this analysis.

Detection
Next Generation Sequencing: Clinical Sensitivity: Unknown. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's clinical/biochemical phenotype.

Analytical sensitivity for sequence variant detection is ~99%.
Copy Number Analysis: The sensitivity and specificity of this method for CNV detection is highly dependent on the size of the event, sequence context and depth of coverage for the region involved. The assay is highly sensitive for CNVs of 500 base pairs or larger and those containing at least 3 exons. Smaller (< 500 base pairs) CNVs and those that involving only 1 or 2 exons may or may not be detected depending on the sequence context, size of exon(s) involved and depth of coverage.

### Specimen Requirements

**Type: DNA, Isolated**

**Specimen Requirements:**
- Microtainer
- 15µg
- 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: 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.