Macrocephaly and Overgrowth Syndromes Panel: Sequencing, CNV Analysis, and Beckwith-Wiedemann Syndrome Methylation Panel

**Test Code:** MM260  
**Turnaround time:** 6 weeks  
**CPT Codes:** 81403 x1, 81404 x1, 81406 x1, 81321 x1, 81401 x1

## Condition Description

Macrocephaly is defined as a head circumference, which is 2 standard deviations larger than the average when matched for age and sex. It refers to an abnormally large head inclusive of the scalp, cranial bone, and intracranial contents. Macrocephaly can arise due to a true enlargement of the brain (megalencephaly) or other conditions such as hydrocephalus and be either syndromic or non-syndromic. The genetic subtypes of macrocephaly include familial forms of macrocephaly, autism, syndromic associations such as PTEN hamartoma syndrome, Noonan syndrome, Sotos syndrome, and metabolic disorders such as glutaric aciduria type 1 and D-2-hydroxyglutaric aciduria.

Reference:

## Genes

`AKT1, AKT2, AKT3, CDKN1C, CUL4B, DNMT3A, EZH2, GLI3, GNAQ, GPC3, H19, LIT1, MED12, MTO1, NFIX, NSD1, PHF6, PIK3CA, PIK3R2, PTEN, RNF135, UPF3B`

## Indications

This test is indicated for:
- Patients with a clinical diagnosis of macrocephaly or other overgrowth 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.

### 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.

### Beckwith-Wiedemann Methylation:
Methylation-specific MLPA (MS-MLPA) is used to test for BWS. One advantage of MS-MLPA is that in addition to detecting DNA methylation abnormalities (epimutations), similar to Southern blot and quantitative methylation sensitive PCR, it also detects deletions and duplications (CNVs) of the 11p15 region. CNVs are estimated to be present in ~10% of patients with BWS. The presence of a CNV can increase the recurrence risk from that of the general population up to a 50% risk. Both methylation and CNVs will be reported from this analysis.

## Detection

### Next Generation Sequencing:
Clinical Sensitivity: Unknown. Pathogenic variants in the promoter region, some pathogenic variants 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.

### Beckwith-Wiedemann Methylation:
Hypomethylation of DMR2 (`Lit1` gene) is expected to detect up to 60-70% of individuals with BWS. Hypermethylation of DMR1 (`H19` gene) is expected to detect an additional 2-13% of individuals with BWS. Therefore, the total detection rate for both DMR1 and DMR2 methylation analysis is estimated to be 62-83%.

## Reference Range

### Beckwith-Wiedemann Methylation:
For DMR1 (`H19` gene), an increase in DNA methylation of greater than two standard deviations above the mean of normal is consistent with BWS. For DMR2 (`Lit1` gene), a decrease in DNA methylation of greater than two standard deviations below the mean of normal is consistent with BWS.
Specimen Requirements

Submit only 1 of the following specimen types

Type: Saliva

Specimen Requirements:
Oragene™ Saliva Collection Kit
Oragene™ 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.

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.

Related Tests

- Macrocephaly: Deletion/Duplication Panel