UAB MEDICAL GENOMICS LABORATORY

Sanger Sequencing of *VHL* Only for Von Hippel-Lindau Syndrome (VHL1)

Ordering Information

Acceptable specimen types:

- Fresh blood sample (3-6 ml EDTA; no time limitations associated with receipt)
- Saliva (OGR-575 DNA Genotek; kits are provided upon request)
- DNA (extracted from lymphocyte cells; a minimum volume of 25µL at 3µg; O.D. of 260:280nm ≥1.8; must be extracted in a CLIA or equivalent certified lab)

Turnaround time:

15 working days

Price and CPT codes:

$650* (USD – institutional/self-pay)

*$400 if mutation is identified during sequencing;

CPT: 81404 and 81403

Z code: ZB68F

Candidates for this test:

Patients seeking confirmation of a clinical diagnosis of VHL with only one of the characteristic manifestations

Specimen shipping and handling:

- Please find acceptable specimen type above.
- All submitted specimens must be sent at room temperature. DO NOT ship on ice.

- Specimens must be packaged to prevent breakage and absorbent material must be included in the package to absorb liquids in the event that breakage occurs. Also, the package must be shipped in double watertight containers (e.g. a specimen pouch + the shipping company's diagnostic envelope).
- To request a sample collection kit, please visit the website or email medgenomics@uabmc.edu to complete the specimen request form.
- Please contact the MGL (via email at medgenomics@uabmc.edu, or via phone at 205-934-5562) prior to sample shipment and provide us with the date of shipment and tracking number of the package so that we can better ensure receipt of the samples.

Required forms:

- Test Requisition Form
- Form for Customs (for international shipments)

Note: Detailed and accurate completion of this document is necessary for reporting purposes. The Medical Genomics Laboratory issues its clinical reports based on the demographic data provided by the referring institution on the lab requisition form. It is the responsibility of the referring institution to provide accurate information. If an amended report is necessary due to inaccurate or illegible documentation, additional reports will be drafted with charge.

Requests for testing may not be accepted for the following reasons:

- No label (patients full name and date of collection) on the specimens
- No referring physician's or genetic counselor's names and addresses
- No billing information
- DNA samples must be extracted in a CLIA or equivalent certified lab

For more information, test requisition forms, or sample collection and mailing kits, please call: 205-934-5562.

Disorder Background

VHL syndrome is an autosomal dominant disorder with a high penetrance (almost complete by 60 years of age) characterized by hemangioblastomas in central nervous system (CNS), retina and other visceral organs. This disorder is also associated with an increased risk of other tumors including clear cell carcinomas of the kidney, pheochromocytoma, renal cysts, pancreatic cystadenoma and pancreatic neuroendocrine tumors. VHL affects ~ 1:35,000 individuals. World-wide prevalence of VHL is approximately 1: 36,000 live births. All ethnic groups and both sexes are affected equally.

The official name for the *VHL* gene is von Hippel-Lindau tumor suppressor, which resides on chromosome 3p25.3. *VHL* gene contains 3 exons and encodes a ~ 4.5 kb mRNA. Loss of function mutations in *VHL* are the only known cause of VHL, and germline *VHL* mutations can be detected in up to 100% of VHL families. Germline mutations are scattered throughout the coding region of the gene. Missense mutations (leading to an amino acid substitution in the *VHL* protein product) are found in 40 % of the families with an identified *VHL* germline mutation. Microdeletions (1-18 bp.), insertions (1-8 bp.), splice site and nonsense mutations, predicted to lead to a truncated protein, are found in approximately 30 % of the families. Large deletions account for one-third of the *VHL* germline mutations, of which approximately 30 % (or some 10 % of all *VHL* germline mutations) are deletions encompassing the entire gene. The *de novo* mutation rate is estimated at 20% and mosaicism may occur in a small percentage of VHL patients.

Test Description

The ***VHL*-only by Sanger** sequencing starts with extraction of DNA from the blood sample of the patient, followed by amplification of three exon fragments. These PCR fragments encompassing the entire *VHL* coding region are hereafter used as the template for direct bi-

directional cycle sequencing (Tier 1). MLPA analysis (Tier 2) is also performed to detect copy number changes, such as multi-exon deletions or duplications and total gene deletions.

REFERENCES available on website.