

Assay Summary

STK11 (LKB1) Gene Mutation Analysis

Peutz-Jeghers syndrome

Synopsis

Peutz-Jeghers syndrome (PJS) is a cancer predisposition syndrome characterized by gastrointestinal polyps and hyperpigmentation of mucocutaneous tissue. Onset of the melanotic macules occurs early in childhood, typically on the lips between the ages of 1 and 5. In some cases, these macules may fade or disappear after puberty. Abdominal complaints generally begin to occur between the ages of 6 and 18, usually as abdominal pain due to intussusception¹. Other less common malignancies associated with PJS are pancreatic, breast, lung, ovary, uterus, cervix, and testicular cancers. The cumulative risk for developing any type of cancer in PJS is 5%, 17%, 31%, 60%, and 85% at ages 30, 40, 50, 60, and 70, respectively².

Mutations in the STK11 gene have been found to cause PJS. Testing for both small alterations (single base pair changes, small insertions/deletions) and for large multi-exon deletions, affords a detection rate of roughly 75% in individuals without a family history. This approaches 95% in individuals with a positive family history of PJS³. Approximately one fourth of these mutations are de novo, and they exhibit incomplete penetrance and variable expressivity.

Indications for testing

Individuals with a clinical diagnosis or suspicion of Peutz-Jeghers syndrome, or at-risk relatives of individuals with a known familial STK11 mutation.

Methodology

STK11 sequencing: All coding exons (1-9) and associated splice site junctions of the STK11 gene are analyzed by direct automated fluorescent sequencing. All sequencing is performed in both the upstream and downstream directions. When a mutation or novel variant is detected, confirmation is carried out on an independent PCR amplification using a second blood prep (when available), and demonstrated in both the upstream and downstream direction.

STK11 MLPA analysis: We have incorporated the SALSA Multiplex Ligation-Dependent Probe Amplification (MLPA) kit that is a rapid, high-throughput technique for copy number quantification⁴, specifically testing for large deletions/duplications for the STK11 gene in PJS. This assay should be considered for patients with PJS where full gene sequencing did not detect a mutation. The P101 STK11 kit contains probes for each of the 10 exons of the STK11 gene on 19p13.3, as well as several probes at close distances telomeric and centromeric of the STK11 gene. The precision and accuracy of the method as a whole has been previously established at >95%, and >99%, respectively.

Performance

Alterations in the STK11 gene are detected in approximately 60% of PJS patients. The sensitivity of DNA sequence analysis for detection of heterozygous point mutations is estimated to be greater than 99%. The specificity of the analysis is estimated to be greater than 98%. Large deletions are estimated to account for approximately 30% of the alterations observed in patients with PJS. The assay sensitivity of deletion detection is >99%.

Limitations

The sequencing analysis will not detect mutations located in regions of the STK11 gene that are not analyzed (non-coding exon regions, intron regions other than the splice junctions, and upstream and downstream regions). The method also will not detect gross genetic alterations including most duplications, inversions, or deletion. Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance.

This MLPA kit is designed to detect deletions / duplications of one or more exons of the STK11 gene. Heterozygous deletions/duplications of probe recognition sequences should give a 35-50% reduced/increased relative peak area of the amplification product of that probe. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe always require confirmation by other methods, when available. MLPA analysis will not detect sequence alterations or inversions.

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 with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes. Keep at 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 3 ccs is sufficient.
- ii) we accept DNA; at least 10 micrograms is required.

- (b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W. A blood sample from the mother maybe required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) 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) 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) [General Cancer Patient Information Form](#): Include a completed General Cancer Patient Information for the proband and a complete pedigree.

Order Codes	CPT Codes	TAT
STK11-SEQ (STK11 gene, full gene sequencing)	83890, 83898(x9), 83894, 83904(x9), 83912	4 wks
STK11-CAS (STK11 gene, targeted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	3 wks
STK11-PD (STK11 gene, known mutation detection, prenatal)	83890, 83898, 83894, 83904, 83912	2 wks
STK11-DEL (STK11 gene, MLPA analysis)	83890, 83896(x13), 83909, 83912	3 wks
STK11-DEL-CAS (STK11 gene, MLPA analysis, known deletions/duplications)	83890, 83896(x13), 83909, 83912	3 wks
STK11-DEL-PD (STK11 gene, MLPA analysis, prenatal)	83890, 83896(x13), 83909, 83912	3 wks

References

1. Westerman A.M. et al. *Lancet*. 1999; 353(9160): 1211–5.
2. Hearle N. et al. *Clin Cancer Res*. 2006; 12(10): 3209-15
3. Aretz S. et al. *Hum Mutat*. 2005; 26(6):513-9
4. Schouten J.P. et al. *Nucleic Acids Res*. 2002 30, e57

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