EXaCT-1 (Whole Exome Sequencing)

TEST DETAILS

Whole Exome Sequencing by Next Generation Sequencing (NGS) has emerged as an efficient strategy to selectively sequence the coding regions of the genome as a more-cost effective alternative to whole genome sequencing. This new approach allows for the identification of numerous gene variations that may yield information about your cancers development, prognosis and any potential targeted therapies that currently exist.

Unlike focused tests, typically called panel sequencing, the EXaCT-1 assay takes an unbiased, exploratory look at more than 20,000 genes in both healthy and malignant cells, allowing molecular pathologists to find alterations in the cancer-development process in unexpected regions of the exome. This type of test, known as whole exome sequencing, can be effective in advanced-stage patients for whom other treatments have failed because it uncovers mutations that the less comprehensive tests miss. In practice, this means, for example, that a patient with bladder cancer whom EXaCT-1 shows to share a mutation associated with breast cancer might benefit from a drug typically prescribed to fight the latter type of tumor.

Specimen Requirements:

EXaCT-1 (Whole Exome Sequencing) requires both a tumor sample AND matched normal sample. In the majority of cases, a blood draw will be scheduled and sent to the lab however in some patients a different type of non-tumorous sample may be required. The clinic team will work with each patient to select the most suitable material.

Tumor Requirements: 15 Unstained slides and 1 HE. The minimum amount of neoplastic cellularity should be at least 20%

Blood Requirements: 2-3ml of Whole Blood in EDTA
Ordering the Test

Please discuss with your oncologist whether ordering this test is right for you.

Oncologist are currently able to order this test through EPIC. The clinic team will then work directly with the patient to schedule a time to collect blood. The pathology department will select the most suitable tumor specimen and send it to the EXaCT-1 laboratory for processing.

Test details and methodology

The EXaCT-1 test uses the HaloPlex Target Enrichment System (Agilent Technologies) for target amplification (357,999 exons/21,522 genes), followed by sequencing on the Illumina system.

Hematoxylin-and-eosin-stained slides are reviewed by a pathologist, and high-density tumor areas are selected for manual macrodissection and DNA extraction. DNA is then extracted from tumors and paired normal tissue, fragmented by restriction enzymes and amplified by PCR following probe-hybridization and circularization of the biotinylated DNA-probe hybrids by ligation. PCR products are then subjected to onboard cluster generation and 100-bp-paired-end sequencing on an Illumina HiSeq 2500 in rapid mode.

Sequencing data is then analyzed by our internally developed informatics pipeline. During the first step of the analysis paired-end reads are aligned to the human genome (reference GRC37/hg19) for each pair of tumor-normal specimens and quality metrics are generated. In the second step, tumor purity is assessed by computational methods and gene variants are called and annotated. Mutations are then classified into three tiers based on their clinical relevance, using publicly available data and our own developed knowledge base. Mutation results are then reviewed and interpreted in the context of the clinical and pathological information by a board-certified molecular pathologist, who also releases the results (sign-out) in the laboratory information management system.
Limitations of the assay

1. The analytical sensitivity of the assay is approximately 10% (with a minimum neoplastic content of 20%), thus, mutations present in a lower percentage of cells may not be identified by this assay. Use of insufficient DNA template can result in low PCR product yields, and sequence signals may fall below detection limits.

2. The human exome is not captured in its entirety, because not all human genes are identified and some genes may not be amendable to capture. Pathogenic mutations located in genes that are non-coding, have corresponding pseudogenes, contain repetitive or high GC-region will not be detected. Information about low coverage regions by this test is provided on our website at: https://profiler17.med.cornell.edu/supplemental_data/IPMWES/HaloPlex_low_coverage_region.xlsx

3. Medium to large indels above 30% of the read length (>60bp) may not be detected due to the short (~200 bp) Illumina reads.

4. The ability of this assay to identify copy number alterations is reduced in cases with low tumor percentage (e.g., less than 50% tumor); in such cases, copy number alteration data (including the apparent absence of copy number alterations) should be interpreted with caution since the findings may not be representative.

5. Any actionable sequence variant detected by this test (or lack of thereof) requires confirmation by an independent testing method before altering clinical management based on the findings.

6. Some regions of genes cannot be fully evaluated for mutations or indels because of lack of sufficient coverage.
Disclaimer

EXaCT-1 was developed and its performance characteristics was determined by the Englander Institute for Precision Medicine/New York Hospital Laboratories. This method has not been cleared by the Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. EXaCT-1 may be used for clinical purpose and should not be regarded as experimental or research only.

The lack of a given genetic alteration in this report does not necessarily indicate the absence of the alteration in the tumor since technical aspects of the assay, including inadequate coverage of some genes, limit the data that can be acquired in some genetic regions. Alterations that occur in the germline are not reported. If a possible pathogenic germline mutation (inherited) is suspected, then counseling by a board certified genetic counselor will be recommended in note.

Contact information:

For further information on this assay or for questions on the results of EXaCT-1 please contact:

Jeffrey Catalano, Operations Manager

http://Jpc2004@med.cornell.edu

212-746-9117

Dr. Wei Song, Laboratory Director

Sow2005@med.cornell.edu

212-746-6419