Test Definition: RETZZ

Multiple Endocrine Neoplasia Type 2 Syndrome, RET, Full Gene Analysis, Varies

Overview

Useful For

Evaluating patients with a personal or family history suggestive of a multiple endocrine neoplasia type 2 (MEN2) or Hirschsprung disease (HSCR)

Establishing a diagnosis of MEN2 or HSCR allowing for targeted cancer surveillance based on associated risks

Identifying variants within genes known to be associated with MEN2 or HSCR allowing for predictive testing of at-risk family members

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in the *RET* gene associated with multiple endocrine neoplasia type 2 and Hirschsprung disease. See Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, familial screening, and genetic counseling for multiple endocrine neoplasia type 2 and Hirschsprung disease.

Special Instructions

- Molecular Genetics: Inherited Cancer Syndromes Patient Information
- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing.

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

For a comprehensive hereditary cancer panel that includes the *RET* gene, consider 1 of the following:
-ENDCP / Hereditary Endocrine Cancer Panel, Varies
-HPGLP / Hereditary Paraganglioma/Pheochromocytoma Panel, Varies

-THYRP / Hereditary Thyroid Cancer Panel, Varies

Testing for the *RET* gene as part of a customized panel is available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies.

Targeted testing for familial variants (also called site-specific or known mutations testing) is available for this gene. For more information see FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

If the reason for testing indicates the *MECP2* gene or Rett Syndrome, order MCP2Z / MECP2 Gene, Full Gene Analysis, Varies. If this test is ordered in this situation, it will be canceled and MCP2Z ordered and performed as the appropriate test.

Specimen Required

Patient Preparation: A previous bone marrow transplant from an allogenic donor will interfere with testing. For instructions for testing patients who have received a bone marrow transplant, call 800-533-1710.
Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Green top (Sodium heparin)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot**.
Specimen Stability Information: Ambient 4 days/Refrigerated 4 days/Frozen 4 days
Additional Information:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for samples received after 4 days and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA is met, the preferred volume of blood must be submitted. Testing may be canceled if DNA requirements are inadequate.

Specimen Type: Saliva
Patient Preparation: Patient **should not** eat, drink, smoke, or chew gum 30 minutes prior to collection.
Supplies: Saliva Collection Kit (T786)
Specimen Volume: 1 Swab
Collection Instructions: Collect and send specimen per kit instructions.
Specimen Stability Information: Ambient (preferred) 30 days/Refrigerated 30 days
Additional information: Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:

-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
2. Molecular Genetics: Inherited Cancer Syndromes Patient Information (T519)
3. If not ordering electronically, complete, print, and send a Oncology Test Request (T729) with the specimen.

Specimen Minimum Volume
Whole blood: 1 mL; Saliva: See Specimen Required

Reject Due To
All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information
Variants in the *RET* proto-oncogene are associated with two distinct and, in rare cases, overlapping clinical syndromes: multiple endocrine neoplasia type 2 (MEN2) and Hirschsprung disease (HSCR).(1)

MEN2:
MEN2 is an autosomal dominant cancer syndrome, which has classically been divided into 3 subtypes: MEN2A, MEN2B, and familial medullary thyroid carcinoma (FMTC). The characteristic features of MEN2A include medullary thyroid carcinoma (MTC), pheochromocytoma, and primary hyperparathyroidism.(1-3) MEN2B is characterized by early-onset MTC, pheochromocytoma, mucosal neuromas, and distinctive facies with enlarged lips. Other features of MEN2B include enlarged nerves of the gastrointestinal tract (ganglioneuromatosis), marfanoid habitus, hypotonia, and corneal nerve thickening.(1-3) FMTC has traditionally been diagnosed in families of MTC in the absence of pheochromocytoma or parathyroid involvement. All MEN2 subtypes are inherited in an autosomal dominant inheritance pattern. The National Comprehensive Cancer Network and the American Thyroid Association provide recommendations regarding the medical management of individuals with MEN2 syndrome.(1,4)

HSCR:
HSCR, also known as aganglionic megacolon, is a congenital disorder of impaired intestinal motility.(1,5,6) Variable lengths of the colon may be affected, resulting in either total aganglionosis, long-segment HSCR, or short-segment HSCR. HSCR affects approximately 1 in 5000 live births and is resolved via surgical intervention.(1,5,6) HSCR can result from chromosome abnormalities, single gene disorders (both syndromic and non-syndromic), a combination of variants in multiple genes, and unknown causes.(1,5,6) However, disease-causing *RET* variants are considered the most common cause of HSCR cases, particularly in families with multiple cases of HSCR and long-segment disease.(1,5,6) It has been reported that up to 50% of familial cases of HSCR and up to 33% of sporadic HSCR cases are due to *RET* germline variants.(1,5,6)

While gain-of-function variants in *RET* are typically associated with MEN2, loss-of-function variants have been reported in patients with HSCR including full or partial *RET* gene deletions.(1) In addition to clearly disease-causing *RET* variants that cause HSCR, additional benign variants in *RET* (which may not be causative in themselves) confer increased susceptibility to HSCR.(1)

Reference Values
An interpretive report will be provided.

Interpretation
All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(7) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions
Clinical Correlations:
Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact the Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances, single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

For detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor at 800-533-1710.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare providers to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(7) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. Incidental findings may include, but are not limited to, results related to the sex chromosomes. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

1. Eng C: Multiple endocrine neoplasia type 2. In: Adam MP, Everman DB, Mirzaa GM, et al, eds. GeneReviews [Internet]. University of Washington, Seattle; 1999. Updated August 10, 2023. Accessed April 26, 2024. Available at www.ncbi.nlm.nih.gov/books/NBK1257/
2. Wells SA Jr, Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015;25(6):567-610. doi:10.1089/thy.2014.0335
3. Moline J, Eng C: Multiple endocrine neoplasia type 2: an overview. Genet Med. 2011;13(9):755-764
4. Haddad RI, Nasr C, Bischoff L, et al. NCCN guidelines insights: Thyroid carcinoma, version 2.2018. J Natl Compr Canc Netw. 2018;16(12):1429-1440
5. Ruiz-Ferrer M, Fernandez RM, Antinolo G, et al. A complex additive model of inheritance for Hirschsprung disease is supported by both RET mutations and predisposing RET haplotypes. Genet Med. 2006;8(11):704-710
6. de Pontual L, Pelet A, Trochet D, et al. Mutations of the RET gene in isolated and syndromic Hirschsprung's disease in

human disclose major and modifier alleles at a single locus. J Med Genet. 2006;43(5):419-423
7. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the *RET* gene, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletion/insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the *RET* gene.

There may be regions of the gene that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences.(Unpublished Mayo method)

The reference transcript for *RET* gene is NM_020975.6. Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report for gene transcript information referenced at the time of testing.

Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

PDF Report

Supplemental

Day(s) Performed

Varies

Report Available

14 to 21 days

Specimen Retention Time

Whole blood: 2 weeks (if available); Extracted DNA: 3 months

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

81406

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
RETZZ	RET Full Gene Analysis	101386-1

Result ID	Test Result Name	Result LOINC® Value
614839	Test Description	62364-5
614840	Specimen	31208-2
614841	Source	31208-2
614842	Result Summary	50397-9
614843	Result	82939-0
614844	Interpretation	69047-9
614845	Resources	99622-3
614846	Additional Information	48767-8
614847	Method	85069-3
614848	Genes Analyzed	48018-6
614849	Disclaimer	62364-5
614850	Released By	18771-6