SAMPLE REPORT

Integrated GENETICS
LabCorp Specialty Testing Group

Inheritest ® NGS, Society Guided Panel

Patient Name:
Referring Physician:
Specimen #:
Patient #:

Client #:
Case #:

DOB: 00/00/0000
Sex:
Lab ID:
Hospital ID:
Specimen Type:

Date Collected: 00/00/0000
Date Received: 00/00/0000

Ethnicity: Not Provided
Indication: Screening

Disease *(Gene)*	RESULTS	INTERPRETATION
Beta hemoglobinopathies, includes sickle cell disease and beta thalassemias *(HBB)*	POSITIVE for one c.19G>A (p.E7K) mutation.	Predicted to be a carrier of hemoglobin C disease. Genetic counseling is recommended. See Additional Clinical Information.
Fragile X syndrome *(FMR1)*	PCR: 32 and 37 repeats.	Negative: not a carrier of a fragile X expansion mutation. This result is not associated with fragile X syndrome.
Spinal muscular atrophy *(SMN1)*	Negative	*SMN1* copy number of two. This result reduces but does not eliminate the risk to be a carrier of SMA. For ethnic specific risk reduction see Information Table.
All other diseases	Negative for the mutations analyzed.	These results reduce, but do not eliminate, the chance to be a carrier. See Information Tables.

Genetic counseling services are available. To access Integrated Genetics Genetic Counselors please call (855)GC-CALLS (855-422-2557).

ADDITIONAL CLINICAL INFORMATION

Beta hemoglobinopathies, includes sickle cell disease and beta thalassemias: Beta hemoglobinopathies, including sickle cell disease and beta thalassemia, are autosomal recessive disorders of red blood cells with variable severity and age of onset. Clinical features of sickle cell disease include chronic hemolytic anemia, pain crises, susceptibility to infection, and organ damage. Features of beta thalassemia typically include mild to severe anemia, hepatosplenomegaly, and skeletal changes. Carriers of beta thalassemia may have mild anemia. Symptoms of other beta hemoglobinopathies depend on the combination of *HBB* gene mutations and can range from mild anemia to severe multi-system disease. (Cao, PMID:20301599; Bender, PMID:20301551). Treatment is primarily supportive and focuses on prevention of complications and management of symptoms. In cases of severe disease, bone marrow or cord blood transplantation can be curative. (Cao, PMID:20301599). Carrier detection should combine clinical information, complete blood count, hemoglobin electrophoresis, and DNA testing (Traeger-Synodinos, PMID: 25052315). Genetic counseling is recommended to discuss the potential clinical and/or reproductive implications of these results, as well as recommendations for testing family members and, when applicable, this individual's partner. If this individual's reproductive partner is also a carrier of a mutation in the same gene the risk for an affected fetus is 25%.

Fragile X syndrome: Fragile X syndrome, also known as Martin-Bell syndrome, is an X-linked disease of intellectual disability with variable severity caused by mutations in the *FMR1* gene. 99% of mutations are expansions of CGG repeat sequences. Rare mutations include missense mutations and gene deletions. Interpretation of repeat expansion results is based on the following ranges: Negative: < 45 repeats; intermediate: 45-54 repeats; premutation: 55-200 repeats with normal methylation pattern; full mutation: >200 repeats with abnormal methylation pattern. Clinical features include mild to severe learning disabilities, autism-like behaviors, developmental delay, increased susceptibility to seizures, and macroorchidism in males. More subtle physical symptoms may include a long, narrow face with prominent ears, joint laxity, and flat feet. Treatment is

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Testing performed at Esoterix Genetic Laboratories, LLC, 3400 Computer Drive, Westborough, MA 01581 Bernice A. Allitto, PhD, FACMG, Laboratory Director 1-800-255-7357

supportive and focuses on educational and behavioral support and management of symptoms. (Santoro, PMID:22017584).

Spinal muscular atrophy: Spinal muscular atrophy (SMA) is an autosomal recessive disease with variable age of onset and severity caused by mutations in the *SMN1* gene. Individuals with one copy of *SMN1* are predicted to be carriers of SMA; those with two or more copies have a reduced carrier risk. Approximately 94% of affected individuals have 0 copies of *SMN1*. In individuals with 0 copies of *SMN1* an increase in the number of copies of the *SMN2* gene correlates with reduced disease severity (Feldkotter M, PMID:11791208). Clinical features of SMA include poor muscle tone, muscle weakness, absence of tendon reflexes, and delayed motor development. In severely affected individuals, abnormal fetal ultrasound findings include congenital joint contractures, polyhydramnios, and decreased fetal movement (Korinthenberg, PMID:9307259). Treatment is supportive.

COMMENTS:

This analysis provides carrier testing by analyzing 12 genes for more than 1200 clinically significant (pathogenic) variants associated with more than 12 autosomal recessive or X-linked diseases. Interpretations and risk calculations, where applicable, are based on the ethnic information and clinical and family relationships provided, as well as the current understanding of the molecular genetics of the conditions tested. References and additional information about the diseases included in Inheritest NGS are available at www.integratedgenetics.com/Inheritest NGS.

The standard of care for Tay-Sachs disease carrier detection in all ethnic groups is enzyme (hexosaminidase A) analysis. For maximum sensitivity and specificity, enzyme analysis should be performed in addition to DNA variant analysis (Schneider, PMID:19876898). If Tay-Sachs enzyme analysis was ordered results are reported separately.

The standard of care for determining carrier status for sickle cell disease and other hemoglobinopathies is to combine information from clinical assessment, complete blood count, hemoglobin electrophoresis, and DNA testing (Traeger-Synodinos, PMID:25052315). If hemoglobin electrophoresis was ordered results are reported separately.

METHOD / LIMITATIONS:

Next generation sequencing (NGS): Genomic regions of interest are selected using the Agilent®SureSelectXT® hybridization capture method for target enrichment and sequenced via the Illumina® next generation sequencing platform. Sequencing reads are aligned with the human genome reference GRCh37/hg19 build. Targeted regions are sequenced to at least 200X mean base coverage with a minimum of 99% of bases at >=20X coverage. Analytical sensitivity is estimated to be >99% for single nucleotide variants and small insertions/deletions (<5 bp). Pathogenic and likely pathogenic variants are confirmed by Sanger sequencing and reported using the numbering and nomenclature recommended by the Human Genome Variation Society (HGVS, http://www.hgvs.org/). Variants of uncertain significance and benign variants are not reported. Variant classification is consistent with ACMG standards and guidelines (Richards, PMID:25741868).

Spinal muscular atrophy: Isolated DNA is amplified by real-time polymerase chain reaction (PCR). The number of copies of exon 7 of *SMN1* is assessed relative to internal standard reference genes. A mathematical algorithm calculates 0, 1, 2 and 3 copies with statistical confidence. In samples with one copy of *SMN1*, primer and probe binding sites are sequenced to rule out variants that could interfere with copy number analysis. In samples with 0 copies of *SMN1, SMN2* copy number is assessed by digital PCR analysis relative to an internal standard reference gene. Copy number analysis cannot detect carriers with either 2 or, very rarely, 3 copies of *SMN1* on one chromosome and no copies of *SMN1* on the other chromosome.

Fragile X syndrome: Isolated DNA is amplified by the polymerase chain reaction (PCR) to determine the size of the CGG repeat within the *FMR1* gene. PCR products are generated using a fluorescence labeled primer and sized by capillary gel electrophoresis. If indicated, Southern blot analysis is performed by hybridizing the probe StB12.3 to EcoRI- and EagI-digested DNA. The analytical sensitivity of both Southern blot and PCR analyses is 99% for expansion mutations in the *FMR1* gene. Reported CGG repeat sizes may vary as follows: +/- one for repeats less than 60, and +/- two to four for repeats in the 60 - 120 range respectively. For repeats greater than 120, the accuracy is +/- 10%.

Limitations: Next generation sequence analysis does not detect germline mosaicism and does not rule out the presence of large chromosomal aberrations (including deletions, insertions and rearrangements), or mutations in regions or genes not included in this test, or possible inter/intragenic interactions between sequence variants. Variant classification and/or interpretation may change over time if more information becomes available. False positive or false negative results may occur for reasons that include: rare genetic variants, pseudogenes, blood transfusions, bone marrow transplantation, somatic or tissue-specific mosaicism, mislabeled samples, or erroneous representation of family relationships.

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INFORMATION TABLES:

SMA risk reductions for individuals with no family history					
Disease (Gene) Reference sequence	**Population**	**Detection Rate**	**Pre-test carrier risk**	**Post-test carrier risk with 2 copy result**	**Post-test carrier risk with 3 copy result**
Spinal muscular atrophy *(SMN1)* NM_000344	African American	70.5%	1 in 72	1 in 130	1 in 4,200
	Ashkenazi Jewish	90.5%	1 in 67	1 in 611	1 in 5,400
	Asian	93.3%	1 in 59	1 in 806	1 in 5,600
	Asian Indian	90.2%	1 in 52	1 in 443	1 in 5,400
	Caucasian	94.8%	1 in 47	1 in 834	1 in 5,600
	Hispanic	90.0%	1 in 68	1 in 579	1 in 5,400
	Mixed or other ethnic background	For counseling purposes, consider using the ethnic background with the most conservative risk estimates			

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Gene-specific risk reductions for individuals with no family history				
Disease (Gene) Reference sequence	**Population**	**Detection Rate**	**Pre-test carrier risk**	**Post-test carrier risk with negative result**
Beta hemoglobinopathy, beta thalassemias *(HBB)* NM_000518	African American East Asian Mediterranean Middle Eastern South Asian Southeast Asian	90% 93% 97% 84% 95% 90%	1 in 75 1 in 50 1 in 20 1 in 30 1 in 20 1 in 30	1 in 741 1 in 700 1 in 634 1 in 182 1 in 381 1 in 291
Beta hemoglobinopathy, hemoglobins C, D, E, and O *(HBB)* NM_000518	African American Asian Asian Indian Middle Eastern Native American Southeast Asian	>99% >99% >99% >99% >99% >99%	1 in 46 1 in 119 1 in 68 1 in 255 1 in 292 1 in 15	Negligible Negligible Negligible Negligible Negligible Negligible
Beta hemoglobinopathy, sickle cell disease *(HBB)* NM_000518	African American Hispanic Middle Eastern Native American	>99% >99% >99% >99%	1 in 14 1 in 183 1 in 360 1 in 176	Negligible Negligible Negligible Negligible
Bloom syndrome *(BLM)* NM_000057	Ashkenazi Jewish	97%	1 in 134	1 in 4434
Canavan disease *(ASPA)* NM_000049	Ashkenazi Jewish	98%	1 in 55	1 in 2700
Cystic fibrosis *(CFTR)* NM_000492	African American Ashkenazi Jewish Asian American Caucasian Hispanic	>81% >97% >55% >93% >78%	1 in 61 1 in 24 1 in 94 1 in 25 1 in 58	1 in 316 1 in 767 1 in 208 1 in 343 1 in 260
Familial dysautonomia *(IKBKAP)* NM_003640	Ashkenazi Jewish	99%	1 in 31	1 in 3000
Fanconi anemia group C *(FANCC)* NM_000136	Ashkenazi Jewish	99%	1 in 100	1 in 9900
Gaucher disease *(GBA)* NM_001005741	Ashkenazi Jewish	98%	1 in 15	1 in 700
Mucolipidosis type IV *(MCOLN1)* NM_020533	Ashkenazi Jewish	96%	1 in 89	1 in 2200
Niemann-Pick disease types A and B *(SMPD1)* NM_000543	Ashkenazi Jewish Worldwide	97% 40%	1 in 116 1 in 250	1 in 3834 1 in 416
Tay-Sachs disease *(HEXA)* NM_000520	Ashkenazi Jewish US French Canadian Worldwide	96%* 47%* 46%*	1 in 27* 1 in 73* 1 in 300*	1 in 650 1 in 136 1 in 554

* Excludes pseudodeficiency alleles

This test was developed and its performance characteristics determined by Esoterix Genetic Laboratories, LLC. It has not been cleared or approved by the Food and Drug Administration.

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Testing performed at Esoterix Genetic Laboratories, LLC, 3400 Computer Drive, Westborough, MA 01581 Bernice A. Allitto, PhD, FACMG, Laboratory Director 1-800-255-7357