Patient Information

Patient Name:	Jane Doe
Date of Birth:	02/02/1975
Gender:	Female
Ethnicity:	Hispanic
Patient ID:	P99457
Medical Record #:	M84555
Collection Kit:	254233-2-C
Accession ID:	40192731
Case File ID:	123456

Test Information

Ordering Physician:	Dr. Goodbirth, M.D. (G123456)
Clinic Information:	Natera, Inc.
Phone:	650 555-1212
Report Date:	09/03/2017
Sample Collected:	09/01/2017
Sample Received:	09/02/2017
Sample Type:	Blood

horizon™
natera carrier screen

CARRIER SCREENING REPORT

ABOUT THIS SCREEN: Horizon™ is a carrier screen for specific autosomal recessive and X-linked diseases. This information can help patients learn their risk of having a child with specific genetic conditions.

ORDER SELECTED: The Horizon **4** panel was ordered for this patient.

FINAL RESULTS SUMMARY: ✓

NEGATIVE FOR 4 OUT OF 4 DISEASES

No pathogenic variants were detected in the genes that were screened. The patient's remaining carrier risk after negative screening results is listed for each disease/gene on the Horizon website at http://www.natera.com/hrzn04/b. Please see the following pages of this report for a comprehensive list of all conditions included on this individual's screen.

Carrier screening is not diagnostic and may not detect all possible pathogenic variants in a given gene.

RECOMMENDATIONS

Individuals who would like to review their Horizon report with a Natera Laboratory Genetic Counselor may schedule a telephone genetic information session by calling 650-249-9090 or visiting naterasession.com. Clinicians with questions may contact Natera at 650-249-9090 or email support@natera.com.

APPROVED BY: J. Dianne Keen-Kim, Ph.D., FACMGG, Senior Laboratory Director

APPROVED BY: Irina Rakova, M.D., CGMBS, Associate Laboratory Director

DISEASES SCREENED

Below is a list of all diseases screened and the result. Certain conditions have unique patient-specific numerical values, therefore, results for those conditions are formatted differently.

Autosomal Recessive

C

Cystic Fibrosis (*CFTR*) **negative**

S

Spinal Muscular Atrophy (*SMN1*)

Negative: SMN1: Two copies; g.27134T>G: absent; the absence of the g.27134T>G variant decreases the chance to be a silent (2+0) carrier.

X-Linked

D

Duchenne/Becker Muscular Dystrophy (*DMD*) **negative**

F

Fragile X Syndrome (*FMR1*)

Negative: 30 and 20 CGG repeats were detected in the FMR1 genes.

Testing Methodology, Limitations, and Comments:

Genomic DNA is isolated utilizing the Promega Maxwell HT 96 gDNA Blood Isolation System.

Genotyping

Analytically difficult regions of the genome such as deletions and duplications >20bp may not be detected in this assay. Sanger sequencing is used to confirm variants prior to reporting. Rarely, novel sequence variants may interfere with NGS read creation, sequence alignment, variant calling and confirmation strategies.

Next Generation Sequencing (NGS)

Genomic DNA is fragmented by sonication, ligated to Illumina P5 and P7 primers, attached to Natera sequencing barcodes (indexes), and PCR amplified. Target enrichment is achieved via hybridization and capture using custom designed probes (NimbleGen, Roche). Massively parallel sequencing is performed using the Illumina NextSeq 500 platform. Regions containing variants within the Promised Variants list are sequenced at a minimum depth of 100X. NGS data is aligned to reference sequence Hg19, followed by deduplication, metric collection and variant calling. Variants are then classified using internal protocols which utilize publicly available databases including but not limited to ENSEMBL, HGMD Pro, ClinGen, ClinVar, 1000G, ESP and gnomAD. SNVs and in/dels identified in NGS are confirmed. This test may not provide detection of certain variants or portions of certain genes due to local sequence characteristics or the presence of closely related pseudogenes. Chimeric or mosaic variants may not be detected with this technology. Gross deletions or duplications and changes from repetitive sequences may not be accurately identified by this methodology.

Sanger Sequencing

Bi-directional Sanger sequencing is performed using target-specific amplicons, BigDye Terminator chemistry, and a ABI 3730 DNA analyzer. In rare cases where unambiguous bi-directional sequencing is difficult or impossible, unidirectional sequence reads may be used for confirmation. Chimeric or mosaic variants may not be detected with this technology.

Copy Number Analysis

MLPA® (Multiplex Ligation-dependent Probe Amplification) probe sets, MRC-Holland, are used to determine copy number of specific targets versus known controls. Each target region is analyzed with two adjacent oligonucleotide probes, which following hybridization, are ligated and used as template for amplification. Resulting amplicons have unique lengths, which are resolved by capillary electrophoresis. False positive or negative results may occur due to rare sequence variants such as small deletions, insertions or mismatches within target regions detected by MLPA probes; any mismatch in the probe's target site can affect the probe signal. MLPA detects the presence of a copy number variation at the covered regions but will not detect copy number changes outside of the detection region of the individual assay and does not define the exact deletion/duplication boundaries.

Spinal Muscular Atrophy

Copy number analysis for *SMN1* is assessed by MLPA. Enhanced SMA testing for the presence or absence of a novel SNP within intron 7 of *SMN1* (g.27134T>G) is assessed using NGS. This SNP is associated with the presence of a *SMN1* duplication allele (Luo et al. 2014, PMID 23788250). Ethnicity-based carrier risk estimates for individuals who are found to carry two *SMN1* copies are listed below.

Ethnicity	**Two SMN1 copies carrier risk before g.27134T>G testing**	**Carrier risk after g.27134T>G testing**	
		g.27134T>G ABSENT	**g.27134T>G PRESENT**
Caucasian	1 in 632	1 in 769	1 in 29
Ashkenazi Jewish	1 in 350	1 in 580	LIKELY CARRIER
Asian	1 in 628	1 in 702	LIKELY CARRIER
African-American	1 in 121	1 in 396	1 in 34
Hispanic	1 in 1061	1 in 1762	1 in 140

Duchenne Muscular Dystrophy

SNVs within the *DMD* gene and promised exons are determined by NGS. MLPA is used to determine copy number of the *DMD* exons. MLPA has lower sensitivity for single exon *DMD* deletions or duplications as contrasted with multi-exon deletion or duplication. The majority of pathogenic DMD-causing variants are multi-exon copy number variants for which this test has a sensitivity of >99%. Natera can only provide limited guidance on the relationship between dystrophin genotypes and expected phenotype.

Fragile X

The CGG repeat region of the *FMR1* 5′-untranslated region is assessed using Asuragen, Inc. AmplideX® FMR1 PCR reagents and capillary electrophoresis. Allele sizes up to 200 repeats are analyzed using a proprietary algorithm. Variances of +/- 3 CGG repeats may occur. This analysis does not detect deletions or point mutations, which comprise less than one percent of the *FMR1* pathogenic variants. Reflex testing for the number of AGG interruptions is performed for CGG repeat sizes between 45 and 90. AGG interruption testing is performed by Asuragen, Inc., 2150 Woodward St. Suite 100 Austin, TX 78744 (CLIA ID: 45D1069375), and will be reported separately.

Categories	CGG Repeat Sizes
Normal	<45
Intermediate	45 – 54
Premutation	55 - 200
Full	>200

Variant Classification

Variants are classified according to ACMG/AMP variant classification guidelines. Only pathogenic or likely pathogenic variants are reported. Benign, likely benign, and variants of uncertain significance are not reported, but may be reported in certain circumstances. Data interpretation is based on our current understanding of genes and variants at the time of reporting. Natera may reclassify variants at certain intervals but may not release updated reports without a specific request made to Natera by the ordering provider. Natera may disclose incidental findings if deemed clinically pertinent to the test performed.

Negative Results

A negative carrier screening result reduces the risk for a patient to be a carrier of a specific disease but does not completely rule out carrier status. Please visit www.natera.com/hrzn04/b for a table of carrier rates, detection rates, residual risks and promised variants/exons per gene. Carrier rates before and after testing vary by ethnicity and assume a negative family history for each disease screened and the absence of clinical symptoms in the patient. Any patient with a family history for a specific genetic disease will have a higher carrier risk prior to testing and, if the disease-causing variant in their family is not included on the test, their carrier risk remains unchanged. Genetic counseling is recommended for patients with a family history of genetic disease so that risk figures based on actual family history can be determined and discussed along with potential implications for reproduction.

Additional Comments

Horizon carrier screening has been developed to identify the reproductive risks for monogenic inherited conditions. Even when one or both members of a couple screen negative for pathogenic variants in a specific gene, the disease risk for their offspring is not zero. There is still a low risk for the condition in their offspring due to a number of different mechanisms that are not detected by Horizon, including but not limited to, pathogenic variant(s) in the tested gene or in a different gene not included on Horizon, pathogenic variant(s) in an upstream regulator, uniparental disomy, de novo mutation(s), or digenic or polygenic inheritance. Infrequent large genetic deletions or duplications are not detected unless they have been specifically targeted for carrier testing.

These tests were developed and their performance characteristics were determined by Natera (CLIA ID: 05D1082992). A portion of the technical component of these tests may have been performed at NSTX, 13011 McCallen Pass, Building A, Suite 110, Austin, TX 78753 (CLIA ID: 45D2093704). The Hex A enzymatic assay, if applicable, was developed and its performance characteristics were determined by Sema4 Genomics 1428 Madison Ave, Atran Bldg, Rm-2-25 New York, NY 10029-6574 (CLIA ID: 33D2097541). These tests have not been cleared or approved by the U.S. Food and Drug Administration (FDA). These analyses generally provide highly accurate information regarding the patient's carrier status; however, there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.