ARUP LABORATORIES
800-522-2787 | aruplab.com
500 Chipeta Way, Salt Lake City, UT 84108-1221
Jonathan R. Genzen, MD, PhD, Chief Medical Officer

Client: Example Client ABC123
123 Test Drive
Salt Lake City, UT 84108
UNITED STATES

Physician: Doctor, Example

Patient: Patient, Example

DOB	Unknown
Gender:	Unknown
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Holoprosencephaly Panel, Sequencing and Deletion/Duplication, Fetal

ARUP test code 2008863

Maternal Contamination Study Fetal Spec	Fetal Cells Single fetal genotype present; no maternal cells present. Fetal and maternal samples were tested using STR markers to rule out maternal cell contamination.
Maternal Contam Study, Maternal Spec	Whole Blood For quality assurance purposes, ARUP Laboratories will confirm the above result at no charge following delivery. Order Confirmation of Fetal Testing and include a copy of the original fetal report (or the mother's name and date of birth) with the test submission. Please contact an ARUP genetic counselor at (800) 242-2787 extension 2141 prior to specimen submission.
Holoprosencephaly Panel Specimen, Fetal	Cultured Amnio
Holoprosencephaly Panel Interp, Fetal	Positive RESULT One pathogenic variant was detected in the ZIC2 gene. PATHOGENIC VARIANT Gene: ZIC2 (NM_007129.5) Nucleic Acid Change: c.1277del; Heterozygous Amino Acid Alteration: p.Pro426ArgfsTer129 Inheritance: Autosomal dominant INTERPRETATION One pathogenic variant, c.1277del; p.Pro426ArgfsTer129, was detected in the ZIC2 gene by massively parallel sequencing in this prenatal sample. Pathogenic ZIC2 variants are associated with holoprosencephaly 5 (MIM: 609637). This result is consistent with a diagnosis of holoprosencephaly. Please refer to the background information included in this report for a list of the genes analyzed, methodology, and limitations of this test. Evidence for variant classification: The ZIC2 c.1277del; p.Pro426ArgfsTer129 variant has been previously identified in a cohort of holoprosencephaly (HPE) patients (Roessler 2009), and is reported in an additional female patient described as having semi-lobar HPE (Solomon

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2010). This variant is absent from the Genome Aggregation Database, indicating it is not a common polymorphism. This variant results in a premature termination codon in the last exon of the ZIC2 gene. While this may not lead to nonsense-mediated decay, it is expected to create a truncated ZIC2, and frameshift variants occurring after this variant have also been identified in HPE patients (Roessler 2009). Based on available information, this variant is considered to be pathogenic.

RECOMMENDATIONS
Genetic consultation is indicated, including a discussion of medical screening and management. At-risk family members, ideally beginning with the parents, should be offered testing for the identified pathogenic ZIC2 variant (Familial Targeted Sequencing, ARUP test code 3005867). Additionally, even if neither parent is found to carry the variant, prenatal diagnosis should be offered in future pregnancies because parental somatic or germline mosaicism for the identified pathogenic variant cannot be excluded.

COMMENTS
Likely benign and benign variants are not reported.
Variants in the following region(s) may not be detected by NGS with sufficient confidence in this sample due to technical limitations:
NONE

REFERENCES
Roessler E et al. The full spectrum of holoprosencephaly-associated mutations within the ZIC2 gene in humans predicts loss-of-function as the predominant disease mechanism. Hum Mutat. 2009 Apr;30(4): E541-54.

Solomon BD et al. Mutations in ZIC2 in human holoprosencephaly: description of a novel ZIC2 specific phenotype and comprehensive analysis of 157 individuals. J Med Genet. 2010 Aug;47(8): 513-524.

This result has been reviewed and approved by [redacted]
BACKGROUND INFORMATION: Holoprosencephaly Panel, Sequencing and Deletion/Duplication, Fetal
CHARACTERISTICS: Holoprosencephaly (HPE) originates from failed midline delineation during early embryonic development and results in partial or complete failure of the prosencephalon to divide into hemispheres. Subtypes of HPE are based on the degree of brain separation and are typically classified as lobar, semilobar, alobar, and middle interhemispheric variant. Microform HPE is an additional subtype that has HPE-related craniofacial anomalies without structural brain defects. The range of clinical findings varies significantly within and between each subtype, including craniofacial characteristics, developmental delay, and neurological impacts. HPE may be an isolated finding or part of a broader syndrome.

EPIDEMIOLOGY: The incidence is 1 in 10,000live births.
CAUSE: 25-60 percent of cases are due to underlying cytogenic abnormalities, including numerical or structural chromosome anomalies and pathogenic copy number variations. Pathogenic germline variants in the single genes account for 25 percent of cases.

INHERITANCE: Dependent on etiology; autosomal dominant for genes tested in this panel

PENETRANCE: Incomplete with highly variable expression

CLINICAL SENSITIVITY: 25 percent

GENES TESTED: CDON, FGFR1*, GLI2, PTCH1, SHH, SIX3, TGIF1, ZIC2*

*One or more exons are not covered by sequencing and/or deletion

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duplication analysis for the indicated gene; see limitations section below.

METHODOLOGY: Probe hybridization-based capture of all coding exons and exon-intron junctions of the targeted genes, followed by massively parallel sequencing. Sanger sequencing was performed as necessary to fill in regions of low coverage and to confirm reported variants that do not meet acceptable quality metrics. A proprietary bioinformatic algorithm was used to detect large (single exon-level or larger) deletions or duplications in the indicated genes. Large deletions/duplications confirmed using an orthogonal exon-level microarray. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY/SPECIFICITY: The analytical sensitivity is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions (indels) from 1-10 base pairs in size. Indels greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced. Deletions of 2 exons or larger are detected with sensitivity greater than 97 percent; single exon deletions are detected with 62 percent sensitivity. Duplications of 3 exons or larger are detected at greater than 83 percent sensitivity. Specificity is greater than 99.9 percent for all variant classes.

LIMITATIONS: A negative result does not exclude a heritable form of holoprosencephaly. This test only detects variants within the coding regions and intron-exon boundaries of the targeted genes. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Regulatory region variants and deep intronic variants will not be identified. Precise breakpoints for large deletions or duplications are not determined in this assay and single exon deletions/duplications may not be detected based on the breakpoints of the rearrangement. The actual breakpoints for the deletion or duplication may extend beyond or be within the exon(s) reported. This test is not intended to detect duplications of 2 or fewer exons in size, though these may be identified. Single exon deletions are reported but called at a lower sensitivity. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations caused by the presence of pseudogenes, repetitive, or homologous regions. This test is not intended to detect low-level mosaic or somatic variants, gene conversion events, complex inversions, translocations, mitochondrial DNA (mtDNA) mutations, or repeat expansions. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Noncoding transcripts were not analyzed.

The following regions are not sequenced due to technical limitations of the assay:

FGFR1 (NM_001354367) exon(s) 18

FGFR1 (NM_001354369) exon(s) 18

FGFR1 (NM_001354370) exon(s) 17

ZIC2 (NM_007129) partial exon(s) 3(Chr13:100637736-100637843)

Single exon deletions/duplications will not be called for the following exons: FGFR1 (NM_001354367, NM_001354369) 18; FGFR1 (NM_001354370) 17

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the U.S. Food and Drug Administration. This test was performed in a CLIA-certified laboratory and is intended for clinical purposes.

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Counseling and informed consent are recommended for genetic testing. Consent forms are available online.

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Maternal Contamination Study Fetal Spec	22-301-105109	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Maternal Contam Study, Maternal Spec	22-301-105109	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Holoprosencephaly Panel Specimen, Fetal	22-301-105109	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Holoprosencephaly Panel Interp, Fetal	22-301-105109	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

END OF CHART

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