CRM ID	: 0000000	Sample Type	: NA
Name	: DUMMY	Date & Time Collected	: DD-MM-YYYY
Sex/Age	: NA	Date & Time Received	: DD-MM-YYYY
Bill. Loc.	: NA	Date & Time Reported	: DD-MM-YYYY
Ref. By	: NA		

CAH DELETION/DUPLICATION REPORT BY MLPA

Specimen Description:

Sample quality is optimum for the test. DNA conc.:25.5 ng/µl
No significant maternal contamination detected.
Maternal cell contamination (MCC) was verified using PCR-based VNTR analysis with a lower limit of detection of 20%.

CYP21A2(CAH) Heterozygous Deletion Detected (Carrier)

Gene	Location	Variant	MLPA probe ratio (Dosage quotient)	Result
CYP21A2	Exon 1	105 nt before ex 1 -113 bp SNP	0.5	Heterozygous Deletion
	Exon 3	13 nt before ex 3, reverse, C-allele at I2G location (CYP21A2:c.293-13C>G)	1.0	Heterozygous Deletion
	Exon 3	13 nt before ex 3, reverse, A-allele at I2G location (CYP21A2:c.293-13C>A)	0	
	Exon 3	350-351 del8bp wildtype	0.5	Heterozygous Deletion
	Exon 4	526-527 I172N wildtype	0.5	Heterozygous Deletion
	Exon 6	721-722 V237E wildtype	0.5	Heterozygous Deletion
	Exon 6	727-726, reverse, M239K wildtype	0.5	Heterozygous Deletion
	Exon 7	927-928 F306+T wildtype	0.5	Heterozygous

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				Deletion

Interpretation Reference:

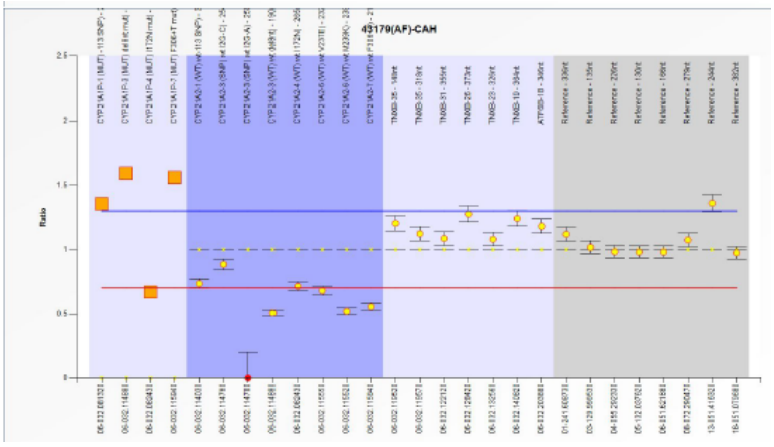
The following DQ values are expected for all probes except those detecting the I2G location in normal sample:

Dosage Quotient [DQ] Distribution	Copy Number Status
DQ = 0	0 copies
0.40 < DQ < 0.65	1 copy
0.80 < DQ < 1.20	2 copies
1.30 < DQ < 1.65	3 copies
1.75 < DQ < 2.15	4 copies
All other values	Ambiguous copy number

Reference for I2G Location :
The CYP21A2 gene has two wildtype alleles at 13 bp before exon 3 location: C and A. These two probes has combined ratio of 1 in case of heterozygous mutant, 0 in case of homozygous mutant and 2 in case of a wild type.

Data from Clinical sample:

Figure 1: MLPA Ratio chart: CAH Probemix



Background:

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Congenital adrenal hyperplasia (CAH) is one of the most common autosomal recessive inherited syndromes. The condition is characterized by impaired cortisol production due to inherited defects in steroid biosynthesis. The clinical consequences of CAH, besides diminished cortisol production, depend on which enzyme is affected and whether the loss of function is partial or complete. In >90% of CAH cases, the affected enzyme is 21-steroid hydroxylase, encoded by the CYP21A2 gene located on chromosome 6.

CAH can be divided into classical and non-classical forms. Clinically, classical CAH occurs as the salt wasting (SW) form with a complete lack of the 21−OH enzyme activity or as the simple virilizing (SV) form with partial impairment of 21−OH enzyme. Most of the inactivating mutations are generated by unequal crossing over or gene conversion between the functional (CYP21A2) gene and a nonfunctional (CYP21A1P) pseudogene. As a result, complete gene deletions/large gene conversions/ 8-bp/single point mutations are manifested with severe phenotypic anomalies in patients with CAH.

Test Methodology:

THE MLPA TECHNOLOGY:

Multiplex ligation-dependent probe amplification (MLPA) is a variation of the multiplex polymerase chain reaction that permits multiple targets to be amplified with only a single primer pair. Each probe consists of two oligonucleotides which recognize adjacent target sites on the DNA. One probe oligonucleotide contains the sequence recognized by the forward primer, the other the sequence recognised by the reverse primer. Only when both probe oligonucleotides are hybridised to their respective targets, can they be ligated into a complete probe. The advantage of splitting the probe into two parts is that only the ligated oligonucleotides, but not the unbound probe oligonucleotides, are amplified. Each complete probe has a unique length, so that its resulting amplicons can be separated and identified by (capillary) electrophoresis. Comparing the peak pattern obtained on a given sample with that obtained on various reference samples, the relative quantity of each amplicon can be determined. This ratio is a measure for the ratio in which the target sequence is present in the sample DNA.

CAH Deletion/Duplication Analysis is based on the MLPA technology (Multiplex Ligation-dependent Probe Amplification) and employs the SALSA® MLPA® probe mixes available from MRC (Holland).

Disclaimer:

- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations,

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the possibility remains that biological changes in that gene or chromosomal region do exist but remain undetected.

- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- Reciprocal exchanges between CYP21A2 and its pseudogene will be missed.
- Sequence changes (SNPs, point mutations) in the target sequence detected by a probe can cause false positive MLPA results.
- Mutations / SNPs close to the ligation site (1-5nt) can reduce or prevent the probe signal by preventing ligation of the two probe oligonucleotides.
- For questions about this report, or for assistance in locating nearby genetic counselling services, please contact the Laboratory: contact@redcliffelabs.com.
- Although all precautions are taken during DNA tests the currently available data indicate that the technical error rate for all types of DNA analysis is approximately 2%. It is important that all clinicians or persons requesting DNA diagnostic tests are aware of these data before acting upon these results.

***References*:**

- *Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation dependent probe amplification. Nucleic Acids Res. 30:e57. Varga RE et al. (2012).*
- *MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. Anal Biochem. 421:799-801.*
- *White PC and Speiser PW (2000). Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev. 21:245-291. Zweers MC et al. (2003).*
- *Haploinsufficiency of TNXB is associated with hypermobility type of Ehlers-Danlos syndrome. Am J Hum Genet. 73:214-217.*

---------- End Of Report ----------

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