Patient Name:	Dummy	**Booking ID:**	XXX
Age:	01 Years	**Sample Type:**	Whole Blood EDTA
Gender:	Male	**Sample collection date:**	14-06-2024
Referring doctor:	Dr.XXX	**Sample receiving date:**	15-06-2024
Test Requested:	Beckwith-Wiedemann Syndrome (BWS)	**Reporting date:**	10-07-2024

Beckwith-Wiedemann Syndrome(BWS) DELETION/DUPLICATION MLPA REPORT

CLINICAL INDICATION/SYMPTOMS

A child brought with complaints of developmentally hemihypertrophy of left side macroglossia.
Clinician's suspicion- Beckwith Wiedemann Syndrome By MLPA

TEST RESULT SUMMARY

No deletion, duplication, and aberrant methylation pattern were detected.

CLINICAL INTERPRETATION

No deletions or duplications were detected in the relevant regions for Beckwith-Wiedemann Syndrome within the detection limits of the Multiplex Ligation-dependent Probe Amplification (MLPA) assay. Additionally, no aberrant methylation was detected in the promoter regions of the CDKN1C, H19, and KCNQ1OT1 genes and the imprinted regions within the detection limits of the Methylation-Specific MLPA (MS-MLPA) assay.

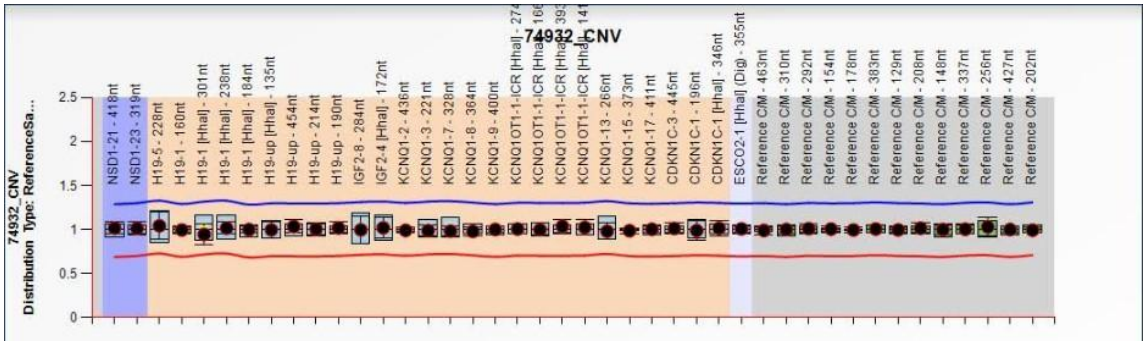

Fig 1: Indicates no significant copy number changes. All probes for the CDKN1C, H19, and KCNQ1OT1 genes, as well as the surrounding regions, fall within the normal range, confirming the absence of deletions or duplications in the assessed areas.

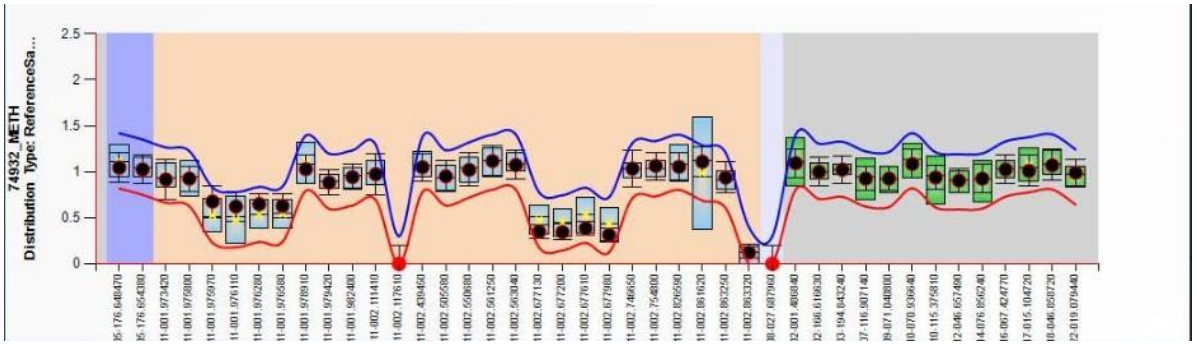

Fig 2: Indicates the CDKN1C, H19, and KCNQ1OT1 gene regions are within the normal range. The probes show no significant copy number changes in these regions, confirming the absence of deletions or duplications. Specifically, for the probes in the H19 and KCNQ1OT1 regions, approximately 45% of the signal remains in the digested reaction, indicating these regions are not methylated. For the gene promoter probes, 0% of the signal remains, indicating these regions are unmethylated. The digestion control probe confirms sufficient HhaI digestion, validating the assay results.

Interpretation Reference:	
Final ratio (FR)	**Copy Number Status**
0.80 < FR < 1.20	NORMAL (identical to reference samples)
FR=0	Homozygous deletion (0 Copy)
0.40 < FR < 0.65	Heterozygous deletion 1 Copy
1.30 < FR < 1.65	Heterozygous duplication 3 Copies
1.75 < FR < 2.15	Heterozygous triplication/homozygous duplication (4 Copies)
All other values	Ambiguous copy number

COMMENT

- ✓ Please correlate clinically.
- ✓ Genetic counseling for accurate interpretation of test results is recommended.
- ✓ Gene sequencing or mutation analysis of targeted region of BWS associated gene is advised to rule out the SNVs, INDELs.
- ✓ For questions about this report, or for assistance in locating nearby genetic counseling services, please contact the Laboratory: geneticcounselors@redcliffelabs.com, ccsupport@redcliffelabs.com.

TEST DESCRIPTION

Beckwith-Wiedemann Syndrome (BWS) is an overgrowth disorder present at birth, with a cumulative incidence rate of approximately 1 in 13,700 live births worldwide. BWS is associated with abnormalities in the 11p15 region, affecting the IGF2 and H19 genes, with imprinting control regions IC1 and IC2 playing crucial roles. The disorder occurs in familial (15%) and sporadic (85%) forms. Identifying deletions, du -plications, or methylation abnormalities in the 11p15 region is essential for clinical management. Recent studies highlight the importance of ' methylation abnormalities in IC1 and IC2 as key diagnostic markers for BWS. Methylation-Specific Multiplex Ligation-dependent Probe Am -plification (MS-MLPA) is used to detect these epigenetic alterations, such as hypomethylation of the H19/IGF2 region (IC1) and hypermethyl -ation of the KvDMR1 region (IC2). This test confirms BWS diagnosis, guides clinical management, and provides vital information for genetic counseling.

METHODOLOGY

Copy number changes in the targeted regions of the CDKN1C, H19, and KCNQ1OT1 genes associated with Beckwith-Wiedemann Syndrome (BWS) were identified using MLPA (Multiplex Ligation-dependent Probe Amplification) probes. Each probe consists of two hemi-probes that bind adjacent to the target sequence, and upon ligation and PCR amplification, generate unique amplicons quantified by capillary electropho -resis. MS-MLPA (Methylation-Specific MLPA) also detects aberrant methylation levels; unmethylated DNA is digested by the HhaI enzyme, preventing amplification, while methylated DNA is protected from digestion, allowing signal generation during analysis.

TEST LIMITATIONS

- ✓ MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect most inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region do exist but remain undetected.
- ✓ A point mutation or polymorphism in the sequence detected by a probe, which results in reduced probe binding efficiency, can also cause a reduction in relative peak area. Therefore, single exon deletions detected by MLPA should always be confirmed by other methods such as multiplex PCR or sequencing.
- ✓ An MS-MLPA probe targets a single specific HhaI site in a CpG island; if methylation is absent for a particular CpG site, this does not nec -essarily mean that the whole CpG island is unmethylated.
- ✓ This test has not been validated by the FDA, NABL, or CAP, and it has been determined by the accrediting bodies that such validation is not required at this time. This report is for research purposes only and is not for use in clinical diagnostic or therapeutic applications.
- ✓ The sensitivity of the MLPA assay may be affected by sample quality and DNA integrity. Poor quality or degraded DNA may result in redu -ced accuracy of the test.
- ✓ The technical and analytical aspects of this report have been performed at NRL, Redcliffe Labs.

DISCLAIMER

- ❖ Test has been performed assuming that the sample received belongs to the above-named individual(s) and that any stated relationships between individuals are accepted as true. It is also assumed that consent for the same was provided after pre- test counseling at the point of collection/referral.
- ❖ The results should be interpreted in the context of the patient's medical evaluation, family history and racial/ethnic background.
- ❖ False negative results may be due to sampling error/errors in sample handling as well as clonal density below the limit of detection. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.
- ❖ The information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician.
- ❖ Patient care and treatment decisions should only be made by the physician after taking into account all relevant information available including but not limited to the patient's condition, family history, findings upon examination, results of other diagnostic tests, and the current standards of care.
- ❖ This report should only be used as an aid and the physician should employ sound clinical judgment in arriving at any decision for patient care or treatment.
- ❖ By providing drug information for the reported diagnosis, Redcliffe Lab Pvt. Ltd. is not guaranteeing that any drug or clinical trial is necessarily appropriate for this patient.
- ❖ Healthcare providers should evaluate and interpret the information provided in this report, along with all other available clinical information about this patient, to determine the best treatment decisions in their own independent medical judgment. Patient management decisions should not be based on a single test, including this one, nor solely on the information contained in this report.

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

Terms and Conditions of Reporting

1. The presented findings in the Reports are intended solely for informational and interpretational purposes by the referring physician or other qualified medical professionals possessing a comprehensive understanding of reporting units, reference ranges, and technological limitations. The laboratory shall not be held liable for any interpretation or misinterpretation of the results, nor for any consequential or incidental damages arising from such interpretation.

2. It is to be presumed that the tests performed pertain to the specimen/sample attributed to the Customer's name or identification. It is presumed that the verification particulars have been cleared out by the customer or his/her representation at the point of generation of said specimen / sample. It is hereby clarified that the reports furnished are restricted solely to the given specimen only.

3. It is to be noted that variations in results may occur between different laboratories and over time, even for the same parameter for the same Customer. The assays are performed and conducted in accordance with standard procedures, and the reported outcomes are contingent on the specific individual assay methods and equipment(s) used, as well as the quality of the received specimen.

4. This report shall not be deemed valid or admissible for any medico-legal purposes.

5. The Customers assume full responsibility for apprising the Company of any factors that may impact the test finding. These factors, among others, includes dietary intake, alcohol, or medication / drug(s) consumption, or fasting. This list of factors is only representative and not exhaustive.