# Test Definition: BWRS

Beckwith-Wiedemann Syndrome/Russell-Silver Syndrome, Molecular Analysis, Varies

## Overview

### Useful For

Confirming a clinical diagnosis of Beckwith-Wiedemann syndrome (BWS) or Russell-Silver syndrome (RSS)

Prenatal diagnosis if there is a high suspicion of BWS/RSS based on ultrasound findings or in families at risk for BWS/RSS

This assay **does not** detect maternal uniparental disomy of chromosome 7 or cytogenetic abnormalities such as translocations or inversions.

### Reflex Tests

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| CULAF | Amniotic Fluid Culture/Genetic Test | Yes | No |
| MATCC | Maternal Cell Contamination, B | Yes | No |
| _STR1 | Comp Analysis using STR (Bill only) | No, (Bill only) | No |
| _STR2 | Add'l comp analysis w/STR (Bill Only) | No, (Bill only) | No |
| CULFB | Fibroblast Culture for Genetic Test | Yes | No |

### Genetics Test Information

This test detects deletions/duplications and determines methylation status in the *BWS*/*RSS* gene cluster. Germline and prenatal testing are available on blood and amniocyte specimens, respectively. Prenatal testing for Beckwith-Wiedemann syndrome and Russell-Silver syndrome cannot be performed on chorionic villus specimens.

### Testing Algorithm

For information see Beckwith-Wiedemann and Russell-Silver Syndromes: Laboratory Approach to Diagnosis

### Special Instructions

- Molecular Genetics: Congenital Inherited Diseases Patient Information
- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)
- Beckwith-Wiedemann and Russell-Silver Syndromes: Laboratory Approach to Diagnosis

### Method Name

Multiplex Ligation-Dependent Probe Amplification (MLPA)

### NY State Available

Yes

## Specimen

### Specimen Type
Varies

### Shipping Instructions
Specimen preferred to arrive within 96 hours of collection.

### Specimen Required
**Patient Preparation**: A previous bone marrow transplant from an allogenic donor will interfere with testing. For information about testing patients who have received a bone marrow transplant, call 800-533-1710.

**Submit only 1 of the following specimens:**

**Specimen Type:** Whole blood
**Container/Tube:** Lavender top (EDTA) or yellow top (ACD)
**Specimen Volume:** 3 mL
**Collection Instructions:**
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot**.
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information.
**Specimen Stability Information:** Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days
**Additional Information:**
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

**Specimen Type**: Cultured fibroblasts
**Source**: Skin
**Container/Tube**: T-25 flask
**Specimen Volume**: 2 Flasks
**Collection Instructions**: Submit confluent cultured fibroblast cells from a skin biopsy.
**Specimen Stability Information**: Ambient (preferred) <24 hours/Refrigerated <24 hours
**Additional Information**:
1. Specimens are preferred to be received within 24 hours of collection. Culture and/or extraction will be attempted for

specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

**Specimen Type**: Tissue biopsy
**Supplies**: Hank's Solution (T132)
**Container/Tube**: Sterile container with sterile Hank's balanced salt solution, Ringer's solution, or normal saline
**Specimen Volume**: 0.5 to 3 cm(3) or larger
**Specimen Stability Information**: Ambient (preferred) <24 hours/Refrigerated <24 hours
**Additional Information**:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

**Specimen Type**: Extracted DNA
**Container/Tube**:
**Preferred**: Screw Cap Micro Tube, 2mL with skirted conical base
**Acceptable**: Matrix tube, 1mL
**Collection Instructions**:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
**Specimen Stability Information**: Frozen (preferred) 1 year/Ambient/Refrigerated
**Additional Information**: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

**Prenatal Specimens**
**Due to its complexity, consultation with the laboratory is required for all prenatal testing; call 800-533-1710 to speak to a genetic counselor.**

**Specimen Type:** Amniotic fluid
**Container/Tube:** Amniotic fluid container
**Specimen Volume:** 20 mL
**Specimen Stability Information:** Ambient (preferred) <24 hours/Refrigerated <24 hours
**Additional Information:** Specimen can be tested only after culture.
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid. An additional 2 to 3 weeks is required to culture amniotic fluid before genetic testing can occur.
**3. All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell

Contamination, Molecular Analysis, Varies on the maternal specimen.

**Specimen Type**: Prenatal cultured amniocytes. This does not include cultured chorionic villi.
**Container/Tube**: T-25 flask
**Specimen Volume**: 2 Flasks
**Collection Instructions**: Submit confluent cultured cells from another laboratory.
**Specimen Stability Information**: Ambient (preferred) <24 hours/Refrigerated <24 hours
**Additional Information**:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing.
3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

### Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
2. Molecular Genetics: Congenital Inherited Diseases Patient Information(T521)
3. If not ordering electronically, complete, print, and send a Neurology Specialty Testing Client Test Request (T732) with the specimen.

### Specimen Minimum Volume

See Specimen Required

### Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Varies | Varies | | |

## Clinical & Interpretive

### Clinical Information

Beckwith-Wiedemann syndrome (BWS) is a disorder characterized by prenatal and/or postnatal overgrowth, neonatal hypoglycemia, congenital malformations, and an increased risk for embryonal tumors. Physical findings are variable and can include abdominal wall defects, macroglossia, and hemihyperplasia. The predisposition for tumor development is associated with specific tumor types such as adrenal carcinoma, nephroblastoma (Wilms tumor), hepatoblastoma, and rhabdomyosarcoma. In infancy, BWS has a mortality rate of approximately 20%.

Current data suggest that the etiology of BWS is due to dysregulation of imprinted genes in the 11p15 region of

chromosome 11, including H19 (maternally expressed), LIT1 (official symbol KCNQ1OT1; paternally expressed), IGF2 (paternally expressed), and CDKN1C (aliases p57 and KIP2; maternally expressed). Expression of these genes is controlled by 2 imprinting centers (IC).

Approximately 85% of BWS cases appear to be sporadic, while 15% of cases are associated with an autosomal dominant inheritance pattern. When a family history is present, the etiology is often due to inherited point alterations in CDKN1C or an unknown cause. The etiology of sporadic cases includes:
-Hypomethylation of imprinting center 2 (IC2) (LIT1): approximately 50% to 60%
-Paternal uniparental disomy of chromosome 11: approximately 10% to 20%
-Hypermethylation of imprinting center 1 (IC1) (H19): approximately 2% to 7%
-Unknown: approximately 10% to 20%
-Point alteration in CDKN1C: approximately 5% to 10%
-Cytogenetic abnormality: approximately 1% to 2%
-Differentially methylated region 1 (DMR1) or DMR2 microdeletion: rare

The clinical presentation of BWS is dependent on which gene in the 11p15 region is involved. The risk for cancer has been shown to be significantly higher in patients with abnormal methylation of IC1 (H19) versus IC2 (LIT1). In patients with abnormal methylation of IC2 (LIT1), abdominal wall defects and overgrowth are seen at a higher frequency.

Russell-Silver syndrome (RSS) is a rare genetic condition with an incidence of approximately 1 in 100,000. RSS is characterized by pre- and postnatal growth retardation with normal head circumference, characteristic facies, fifth finger clinodactyly, and asymmetry of the face, body, and/or limbs. Less commonly observed clinical features include cafe au lait spots, genitourinary anomalies, motor, speech, cognitive delays, and hypoglycemia. Although clinical diagnostic criteria have been developed, it has been demonstrated that many patients with molecularly confirmed RSS do not meet strict clinical diagnostic criteria for RSS. Therefore, most groups recommend a relatively low threshold for considering molecular testing in suspected cases of RSS.

Russell-Silver syndrome is a genetically heterogeneous condition that is associated with genetic and epigenetic alterations at chromosome 7 and the chromosome 11p15.5 region. The majority of cases of RSS are sporadic, although familial cases have been reported. The etiology of sporadic cases of RSS includes:
-Hypomethylation of IC1 (H19): approximately 30% to 50%
-Maternal uniparental disomy (UPD) of chromosome 7: approximately 5% to 10%
-11p15.5 duplications: rare
-Chromosome 7 duplications: rare
*Note that this test does not detect chromosome 7 UPD. However, testing is available; order UNIPD / Uniparental Disomy, Varies.

The clinical phenotype of RSS has been associated with the specific underlying molecular etiology. Patients with hypomethylation of IC1 (H19) are more likely to exhibit "classic" RSS phenotype (ie, severe intrauterine growth retardation, postnatal growth retardation, and asymmetry), while patients with maternal UPD7 often show a milder clinical phenotype. Despite these general genotype-phenotype correlations, many exceptions have been reported.

Methylation abnormalities of IC1 (H19) and IC2 (LIT1) can be detected by methylation-sensitive multiple ligation-dependent probe amplification. While testing can determine methylation status, it does not identify the

mechanism responsible for the methylation defect (such as paternal uniparental disomy or cytogenetic abnormalities). Hypomethylation of IC2 (LIT1) is hypothesized to silence the expression of a number of maternally expressed genes, including CDKN1C. Hypermethylation of IC1 is hypothesized to silence the expression of H19, while also resulting in overexpression of IGF2. Absence of CDKN1C and H19 expression, in addition to overexpression of IGF2, is postulated to contribute to the clinical phenotype of BWS. Hypomethylation of IC1 is hypothesized to result in overexpression of H19 and underexpression of the IGF2, which is thought to contribute to the clinical phenotype of RSS.

### Reference Values

An interpretive report will be provided.

### Interpretation

The interpretive report includes an overview of the findings as well as the associated clinical significance.

### Cautions

In addition to disease-related probes, the multiple ligation-dependent probe amplification technique utilizes probes localized to other chromosomal regions as internal controls. In certain circumstances, these control probes may detect other diseases or conditions for which this test was not specifically intended. Results of the control probes are not normally reported. However, in cases where clinically relevant information is identified, the ordering physician will be informed of the result and provided with recommendations for any appropriate follow-up testing.

Rare variants (ie, polymorphisms) exist that could lead to false-negative or false-positive results. If results obtained do not match the clinical findings, additional testing should be considered.

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Errors in the interpretation of results may occur if information given is inaccurate or incomplete.

Methylation status cannot be assessed on chorionic villus specimens.

This assay does not detect maternal uniparental disomy of chromosome 7 or cytogenetic abnormalities such as translocations or inversions.

### Supportive Data

Normal methylation index was derived by studying 150 normal individuals. For 65 patients referred for Beckwith-Wiedemann syndrome testing, results of this multiple ligation-dependent probe amplification (MLPA) assay were compared to a Southern blot method. Results were concordant for 64 of 65 specimens. In one specimen, a deletion was identified by MLPA that was not detected by the Southern blot method. For 55 patients referred for Russell-Silver syndrome testing, results of this MLPA assay were compared to *H19* Southern blot. Results were concordant for 53 of 55 specimens. Two amniotic fluid specimens were positive for a *H19* hypomethylation defect by Southern blot that were not detected by MLPA.

### Clinical Reference

1. DeBaun MR, Niemitz EL, McNeil DE, Brandenburg SA, Lee MP, Feinberg AP. Epigenetic alterations of *H19* and *LIT1* distinguish patients with Beckwith-Wiedemann Syndrome with cancer and birth defects. Am J Hum Genet. 2002;70(3):604-611

2. Choufani S, Shuman C, Weksberg R. Beckwith-Wiedemann Syndrome. Am J Med Genet C Semin Med Genet.

2010;154C(3):343-354
3. Wakeling EL. Silver-Russell syndrome. Arch Dis Child. 2011;96(12):1156-1161
4. Eggermann T, Begemann M, Binder G, Spengler S. Silver-Russell syndrome: genetic basis and molecular genetic testing. Orphanet J Rare Dis. 2010;5:19
5. Priolo M, Sparago A, Mammi C, Cerrato F, Lagana C, Riccio A. MS-MLPA is a specific and sensitive technique for detecting all chromosome 11p15.5 imprinting defects of BWS and SRS in a single-tube experiment. Eur J Hum Genet. 2008;16(5):565-571
6. Brioude F, Kalish JM, Mussa A, et al. Expert consensus document: Clinical and molecular diagnosis, screening and management of Beckwith-Wiedemann syndrome: an international consensus statement. Nat Rev Endocrinol. 2018;14(4):229-249. doi:10.1038/nrendo.2017.166
7. Wakeling EL, Brioude F, Lokulo-Sodipe O, et al. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinol. 2017;13(2):105-124. doi:10.1038/nrendo.2016.138

## Performance

### Method Description
Methylation-sensitive multiple ligation-dependent probe amplification is utilized to test for the presence of large deletions, duplications, and methylation defects in the imprinting center 1 (IC1) (*H19*) and IC2 (*LIT1*) critical regions on chromosome 11p15.(Unpublished Mayo method)

### PDF Report
No

### Day(s) Performed
Varies

### Report Available
10 to 14 days

### Specimen Retention Time
Whole Blood: 30 days (if available); Extracted DNA: 3 months

### Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

## Fees & Codes

### Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

## Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

## CPT Code Information

81401-H19 (imprinted maternally expressed transcript [non-protein coding]) (eg, Beckwith-Wiedemann syndrome), methylation analysis
81401-KCNQ1OT1 (KCNQ1 overlapping transcript 1 [non-protein coding]) (eg, Beckwith-Wiedemann syndrome) methylation analysis
88233-Tissue culture, skin or solid tissue biopsy (if appropriate)
88240-Cryopreservation (if appropriate)
88235-Tissue culture for amniotic fluid (if appropriate)
81265-Comparative analysis using Short Tandem Repeat (STR) markers; patient and comparative specimen (eg, pre-transplant recipient and donor germline testing, post-transplant non-hematopoietic recipient germline [eg, buccal swab or other germline tissue sample] and donor testing, twin zygosity testing or maternal cell contamination of fetal cells (if appropriate)

## LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| BWRS | BWS/RSS Molecular Analysis | In Process |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 52845 | Result Summary | 50397-9 |
| 52846 | Result | 82939-0 |
| 52847 | Interpretation | 69047-9 |
| 52848 | Reason for Referral | 42349-1 |
| 52849 | Specimen | 31208-2 |
| 52850 | Source | 31208-2 |
| 52851 | Released By | 18771-6 |