MAYO CLINIC LABORATORIES

# Test Definition: AGDD

Alpha Globin Cluster Locus Deletion/Duplication, Varies

## Overview

### Useful For

Diagnosis of alpha-thalassemia

Carrier screening for individuals from high-risk populations for alpha-thalassemia

This test is **not useful for** diagnosis or confirmation of beta-thalassemia or hemoglobinopathies.

### Reflex Tests

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

### Genetics Test Information

This test is for genetic deletions and duplications only.

### Testing Algorithm

**For prenatal specimens only:**

If an amniotic fluid (nonconfluent cultured cells) is received, amniotic fluid culture will be added and performed at an additional charge.

If a chorionic villus specimen (nonconfluent cultured cells) is received, fibroblast culture for genetic test will be added and performed at an additional charge.

For any prenatal specimen that is received, maternal cell contamination studies will be added and performed at an additional charge. **A maternal whole blood specimen is required to perform this test**.

### Special Instructions

- Molecular Genetics: Congenital Inherited Diseases Patient Information
- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)

### Method Name

Polymerase Chain Reaction (PCR)/Quantitative Polymerase Chain Reaction (qPCR)/Multiplex Ligation-Dependent Probe Amplification (MLPA)

### NY State Available
Yes

## Specimen

### Specimen Type
Varies

### Ordering Guidance
Sequence variants, other than the alpha T-Saudi and hemoglobin constant spring alterations, are **not** detected by this assay. For detection of single point and other nondeletion variants, order WASEQ / Alpha Globin Gene Sequencing, Varies, if clinically indicated.

Hemoglobin electrophoresis is **recommended** prior to this test to exclude other diagnoses.

### Additional Testing Requirements
All prenatal specimens must be accompanied by a maternal blood specimen. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen. This must be a different order number than the prenatal specimen.

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

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

**Specimen Type:** Whole blood
**Container/Tube:**
**Preferred:** Lavender top (EDTA) or yellow top (ACD)
**Acceptable:** Green top (sodium heparin)
**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**: Extracted DNA
**Container/Tube**:
**Preferred**: Screw Cap Micro Tube, 2 mL with skirted conical base
**Acceptable**: Matrix tube, 1 mL
**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 will only be tested 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 are 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:** Confluent cultured amniocytes
**Container/Tube:** T-25 flask
**Specimen Volume:** 2 Full flasks
**Collection Instructions:** Submit confluent cultured amniocytes 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.

**Specimen Type:** Chorionic villi
**Container/Tube:** 15-mL tube containing 15 mL of transport media
**Specimen Volume:** 20 mg
**Specimen Stability Information:** Ambient (preferred) <24 hours/Refrigerated <24 hours
**Additional Information:** Specimen will only be tested after culture.
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 are required to culture fibroblasts 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**: Cultured chorionic villi
**Container/Tube**: T-25 flasks
**Specimen Volume**: 2 Full 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 Benign Hematology Test Request Form (T755) 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

Thalassemias are a group of inherited conditions characterized by decreased synthesis of one or more of the globin chains, resulting in an imbalance in the relative amounts of the alpha and beta chains. The excess normal chains precipitate in the cell, damaging the membrane and leading to premature red blood cell destruction. Additionally, the defect in hemoglobin synthesis produces a hypochromic, microcytic anemia. The frequency of thalassemia is due to the protective advantage against malaria that it gives carriers. Consequently, thalassemias are prevalent in populations from equatorial regions in the world where malaria is endemic.

Alpha-thalassemia is caused by decreased synthesis of alpha-globin chains. Four alpha-globin genes are normally present (2 on each chromosome 16). One, 2, 3, or 4 alpha-globin genes may be deleted or, less commonly, contain variants. Deletions account for approximately 90% of disease-causing alleles in alpha thalassemia. Phenotypically, these deletions result in 4 categories of disease expression:
-Deletion of 1 alpha-chain: Silent carrier state, with a normal phenotype
-Deletion of 2 alpha-chains: Alpha-thalassemia trait (alpha-1 thalassemia), with mild hematologic changes but no major clinical difficulties
-Deletion of 3 alpha-chains: Hemoglobin H disease, which is extremely variable but usually includes anemia due to hemolysis, jaundice, and hepatosplenomegaly
-Deletion of all 4 alpha-chains: Hemoglobin Barts hydrops fetalis and, almost invariably, in utero fetal demise or early after birth if left untreated. Samples with protein effects of intrauterine transfusion are increasingly common.

Less frequently, alpha-thalassemia results from single point alterations, such as hemoglobin Constant Spring (*HBA2*: c.427T >C).

Alpha-thalassemia occurs in all ancestral groups but is especially common in individuals of Southeast Asian and African ancestry. It is also frequent in individuals of Mediterranean ancestry. The carrier frequency is estimated to be 1 in 20 for Southeast Asians, 1 in 30 for African, and 1 in 30 to 1 in 50 for individuals of Mediterranean ancestry. Both deletional and nondeletional (caused by sequence alterations) forms of alpha-thalassemia are found in individuals with Mediterranean ancestry. Those of Arab ancestry can carry a fairly common sequence alteration, called alpha-T-Saudi. Deletions in cis (two deletions on the same chromosome) are rare in African or Mediterranean populations but are prevalent in Asian populations. Couples in which both partners carry deletions in cis are at risk of having a child with hemoglobin H disease or hemoglobin Bart hydrops fetalis syndrome.

### 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

Rare alterations (ie, polymorphisms) exist that could lead to false-negative or false-positive results. If the 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.

### Clinical Reference

1. Harteveld CL, Voskamp A, Phylipsen M, et al. Nine unknown rearrangements in 16p13.3 and 11p15.4 causing alpha- and beta-thalassaemia characterised by high resolution multiplex ligation-dependent probe amplification. J Med Genet. 2005;42(12):922-931. doi:10.1136/jmg.2005.033597
2. Harteveld CL, Higgs DR. Alpha-thalassaemia. Orphanet J Rare Dis. 2010;5:13. doi:10.1186/1750-1172-5-13
3. Bunn HF, Forget BG. Hemoglobin: Molecular, Genetic and Clinical Aspects. 2nd ed. WB Saunders Company; 1986
4. Weatherall DJ, Higgs DR, Clegg JB, Hill AS, Nicholls R. Heterogeneity and origins of the alpha-thalassemias. Birth Defects Orig Artic Ser. 1987;23(5A):3-14
5. Musallam KM, Cappellini MD, Coates TD, et al. Alpha-thalassemia: A practical overview. Blood Rev. 2024;64:101165. doi:10.1016/j.blre.2023.101165

## Performance

### Method Description

This test is a direct variant analysis assay. Deletions and duplications within the alpha-globin locus are identified by a multiplex ligation-dependent probe amplification assay. Thirty-three probes that hybridize throughout the alpha-globin locus from the HS40 promoter region through the 3'HVR region are utilized to maximize the information needed to map the approximate location of nearly all DNA deletions that occur. A quantitative polymerase chain reaction (PCR)-based assay is used to detect the presence of hemoglobin Constant Spring (*HBA2*: c.427T>C) and alphaT-Saudi (*HBA2*: c.*94A>G) point alterations. In addition, a PCR-based assay is used to detect the presence of the alpha-3.7 and alpha-4.2 deletions.(Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 2002;30[12]:e57. doi:10.1093/nar/gnf056)

### PDF Report

No

### Day(s) Performed

Varies

### Report Available

9 to 13 days

### Specimen Retention Time

Whole blood: 28 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

81269
88235-Tissue culture for amniotic fluid (if appropriate)
88240-Cryopreservation (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 |
|---|---|---|
| AGDD | Alpha Globin Cluster Locus Del/Dup | 90040-7 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 620986 | Result Summary | 50397-9 |
| 620987 | Result | 82939-0 |
| 620988 | Interpretation | 69047-9 |
| 620989 | Additional Information | 48767-8 |
| 620990 | Specimen | 31208-2 |
| 620991 | Source | 31208-2 |
| 620992 | Method | 85069-3 |
| 621803 | Disclaimer | 62364-5 |
| 620993 | Released By | 18771-6 |