# Test Definition: CDGF

Celiac Disease Gluten-Free Cascade, Serum and Whole Blood

## Overview

### Useful For

Evaluating patients suspected of having celiac disease who are currently (or were recently) on a gluten-free diet

### Profile Information

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| CELI2 | HLA-DQ Typing | Yes, (Order CELI) | Yes |
| CDGF1 | Celiac Disease Interpretation | No | Yes |

### Reflex Tests

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| TTGA | Tissue Transglutaminase Ab, IgA, S | Yes | No |
| DAGL | Gliadin(Deamidated) Ab, IgA, S | Yes | No |
| DGGL | Gliadin(Deamidated) Ab, IgG, S | Yes | No |
| TTGG | Tissue Transglutaminase Ab, IgG, S | Yes | No |
| IGA | Immunoglobulin A (IgA), S | Yes | No |

### Testing Algorithm

If *HLA-DQ* typing is positive or equivocal for *DQ2* or *DQ8*, then IgA, tissue transglutaminase IgA and IgG, and deamidated gliadin IgA and IgG antibody testing will be performed at an additional charge.

The following algorithms are available:
-Celiac Disease Gluten-Free Cascade Test Algorithm.
-Celiac Disease Routine Treatment Monitoring Algorithm

### Special Instructions

- Celiac Disease Gluten-Free Cascade Test Algorithm
- Celiac Disease Routine Treatment Monitoring Algorithm

### Method Name

CELI2: Polymerase Chain Reaction (PCR)/Sequence-Specific Oligonucleotide Probe (SSO)
CDGF1: Technical Interpretation

**NY State Available**
Yes

## Specimen

### Specimen Type
Whole Blood ACD-B
Serum

### Ordering Guidance
This cascade **should not be used** in patients for whom human leukocyte antigen (HLA) DQ2/DQ8 typing has already been performed. For individuals who are positive for either DQ2 and/or DQ8, CDSP / Celiac Disease Serology Cascade, Serum should be ordered to assess for the presence of autoantibodies associated with celiac disease. For individuals who are negative for DQ2 and DQ8, no further testing is necessary as a diagnosis of celiac disease is unlikely.

Cascade testing is recommended for celiac disease. Cascade testing ensures that testing proceeds in an algorithmic fashion. The following cascades are available; select the appropriate one for your specific patient situation.
-CDCOM / Celiac Disease Comprehensive Cascade, Serum and Whole Blood: Complete testing including HLA DQ
-CDSP / Celiac Disease Serology Cascade, Serum: Complete serology testing excluding HLA DQ
-CDGF / Celiac Disease Gluten-Free Cascade, Serum and Whole Blood: For patients already adhering to a gluten-free diet

To order individual tests, see Celiac Disease Diagnostic Testing Algorithm

### Specimen Required
**Both whole blood and serum are required.**

**Specimen Type:** Whole Blood
**Container/Tube:**
**Preferred:** Yellow top (ACD solution A or B)
**Acceptable**: No additional anticoagulants are acceptable.
**Specimen Volume:** 6 mL
**Collection Instructions:** Send whole blood in original tube. **Do not aliquot**.

**Specimen Type:** Serum
**Supplies:** Sarstedt Aliquot Tube, 5 mL (T914)
**Collection Container/Tube:**
**Preferred:** Serum gel
**Acceptable:** Red top
**Submission Container/Tube:** Plastic vial
**Specimen Volume:** 2 mL
**Collection Instructions:** Centrifuge and aliquot serum into plastic vial

### Forms

If not ordering electronically, complete, print, and send Gastroenterology and Hepatology Test Request (T728) with the specimen

### Specimen Minimum Volume

Blood: 3 mL
Serum: 1.5 mL

### Reject Due To

| | |
|---|---|
| Gross hemolysis | Reject |
| Gross lipemia | Reject |
| Gross icterus | OK |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Whole Blood ACD-B | Refrigerated (preferred) | | |
| | Ambient | | |
| Serum | Refrigerated (preferred) | 21 days | |
| | Frozen | 21 days | |

## Clinical & Interpretive

### Clinical Information

Celiac disease (gluten-sensitive enteropathy, celiac sprue) results from an immune-mediated inflammatory process following ingestion of wheat, rye, or barley proteins that occurs in genetically susceptible individuals.(1) The inflammation in celiac disease occurs primarily in the mucosa of the small intestine, which leads to villous atrophy. Common clinical manifestations related to gastrointestinal inflammation include abdominal pain, malabsorption, diarrhea, and constipation. Clinical symptoms of celiac disease are not restricted to the gastrointestinal tract. Other common manifestations of celiac disease include failure to grow (delayed puberty and short stature), iron deficiency, recurrent fetal loss, osteoporosis, chronic fatigue, recurrent aphthous stomatitis (canker sores), dental enamel hypoplasia, and dermatitis herpetiformis. Patients with celiac disease may also present with neuropsychiatric manifestations, including ataxia and peripheral neuropathy, and are at increased risk for developing non-Hodgkin lymphoma. The disease is also associated with other clinical disorders, including thyroiditis, type I diabetes mellitus, Down syndrome, and IgA deficiency.

Individuals with family members who have celiac disease are at increased risk of developing the disease.(2) Genetic susceptibility is related to specific human leukocyte antigen (HLA) markers. More than 97% of individuals with celiac disease in the United States have *DQ2* and/or *DQ8* HLA markers, compared with approximately 40% of the general population. For this reason, *HLA-DQ2* and *HLA-DQ8* are considered genetic risk factors for celiac disease and are required, but not sufficient, for the disease process to occur. HLA testing is not required for diagnosis in all cases but can

be useful in situations where histology and serology are discrepant or for individuals who have started a gluten free diet before evaluation.(3)

A definitive diagnosis of celiac disease requires a duodenal biopsy demonstrating villous atrophy.(3) Given the invasive nature and cost of the biopsy, serologic and genetic laboratory tests may be used to identify individuals with a high probability of having celiac disease. Because no single laboratory test can be relied upon completely to establish a diagnosis of celiac disease, individuals with positive laboratory results should be referred for small intestinal biopsy, thereby decreasing the number of unnecessary invasive procedures. In terms of serology, celiac disease is associated with a variety of autoantibodies, including endomysial, tissue transglutaminase (tTG), and deamidated gliadin antibodies.(4) Although the IgA isotype of these antibodies usually predominates in celiac disease, individuals may also produce IgG isotypes, particularly if the individual is IgA deficient. The most sensitive and specific serologic test is tTG IgA isotype in individuals who produce sufficient total IgA. For individuals who are IgA deficient, testing for tTG and deamidated gliadin IgG antibodies is required.

A recent multi-cohort international study found that a tTG IgA titer of 10 or more times the upper limit of normal (ULN) had a positive predictive value of 95% in an adult population.(5) In addition, several prospective studies have shown that a biopsy free approach to celiac disease diagnosis may be possible in children with a tTG titer 10 or more times the ULN who meet certain criteria.(6-9) Given this evidence, the American College of Gastroenterology now suggests that a positive tTG IgA result greater than 10times the ULN with a positive endomysial antibody in a separate blood sample may be sufficient for a diagnosis celiac disease in children.(3)

The treatment for celiac disease is maintenance of a gluten-free diet. In most patients who adhere to this diet, concentrations of associated autoantibodies decline, which is sometimes also accompanied by reconstitution of the small intestinal villi. In most patients, an improvement in clinical symptoms is observed. For evaluation purposes, all serologic tests ordered for the diagnosis of celiac disease should be performed while the patient is on a gluten-containing diet. Once a patient has initiated the gluten-free diet, serologic testing may be repeated to assess the response to treatment. In some patients, antibody titers may take up to 1 year to normalize. Persistently elevated results suggest poor adherence to the gluten-free diet or the possibility of refractory celiac disease.

It should be noted that HLA typing is not required to establish a diagnosis of celiac disease. Consider ordering CDSP / Celiac Disease Serology Cascade, Serum if HLA typing is not desired or has been previously performed.

For the recommended approach to a patient suspected of celiac disease, see Celiac Disease Diagnostic Testing Algorithm.

For monitoring the patient's response to treatment, see Celiac Disease Routine Treatment Monitoring Algorithm.

## Reference Values

HLA-DQ TYPING

Presence of *HLA-DQ2* or *HLA-DQ8* alleles associated with celiac disease

## Interpretation

*HLA-DQ* Typing:

Approximately 90% to 95% of patients with celiac disease have the *HLA-DQ2* allele; most of the remaining patients with

celiac disease have the *HLA-DQ8* allele. Individuals who do not carry either of these alleles are unlikely to have celiac disease. For these individuals, no further serologic testing is required. However, individuals with these alleles may not, during their lifetime, develop celiac disease. Therefore, the presence of *DQ2* or *DQ8* does not conclusively establish a diagnosis of celiac disease. For individuals with *DQ2* and/or *DQ8* alleles, in the context of positive serology and compatible clinical symptoms, small intestinal biopsy is recommended.

Immunoglobulin A:
Total IgA levels below the age-specific reference range suggest either a selective IgA deficiency or a more generalized immunodeficiency. For individuals with a low or high IgA level, additional clinical and laboratory evaluation is recommended. Some individuals may have a partial IgA deficiency in which the IgA levels are detectable but fall below the age-adjusted reference range. For these individuals, both IgA and IgG isotypes for tissue transglutaminase (tTG) and deamidated gliadin antibodies are recommended for the evaluation of celiac disease.

tTG IgA/IgG Antibodies:
Individuals positive for tTG antibodies of the IgA and/or IgG isotype may have celiac disease, and a small intestinal biopsy is recommended. For individuals with selective IgA deficiency, testing for tTG antibodies of the IgG isotype is indicated. In these individuals, a positive tTg IgG antibody result suggests a diagnosis of celiac disease. However, just as with the tTG IgA antibody, a biopsy should be performed to confirm the diagnosis. Negative tTG IgA and/or IgG antibody serology does not exclude a diagnosis of celiac disease, as antibody levels decrease over time in patients who have been following a gluten-free diet.

Deamidated Gliadin IgA/IgG Antibodies:
Positivity for deamidated gliadin antibodies of the IgA and/or IgG isotype is suggestive of celiac disease, and a small intestinal biopsy is recommended. For individuals with selective IgA deficiency, testing for deamidated gliadin antibodies of the IgG isotype is indicated. In these individuals, a positive deamidated gliadin IgG antibody result suggests a diagnosis of celiac disease. However, just as with the deamidated gliadin IgA antibody, a biopsy should be performed to confirm the diagnosis. Negative deamidated gliadin IgA and/or IgG antibody serology does not exclude a diagnosis of celiac disease, as antibody levels decrease over time in patients who have been following a gluten-free diet.

## Cautions

This cascade should not be solely relied upon to establish a diagnosis of celiac disease. It should be used to identify patients who have an increased probability of having celiac disease and for whom a small intestinal biopsy is recommended.

This cascade is designed for use in patients who have already instituted or recently discontinued a gluten-free diet. For patients who are not following a gluten-free diet, CDCOM / Celiac Disease Comprehensive Cascade, Serum and Whole Blood is the preferred test.

See individual tests for analyte specific cautions.

## Clinical Reference

1. Rubin JE, Crowe SE. Celiac disease. Ann Int Med. 2020;172(1):ITC1-ITC16
2. Lebwohl B, Rubio-Tapia A. Epidemiology, presentation, and diagnosis of celiac disease. Gastroenterology. 2021;160(1):63-75

3. Rubio-Tapia A, Hill, ID, Semrad, C, Kelly CP, Greer, KB, Limketkai, BN, Lebwohl B, American College of Gastroenterology Guidelines update: Diagnosis and management of celiac disease. Am J Gastroenterol. 2023;118(1):59-76. doi:10.14309/ajg.0000000000002075
4. Penny HA, Raju SA, Sanders DS. Progress in the serology-based diagnosis and management of adult celiac disease. Exp Rev Gastroenterol Hepatol. 2020;14(3):147-154
5. Penny HA, Raju SA, Lau MS, Marks LJ, et.al. Accuracy of a no-biopsy approach for the diagnosis of coeliac disease across different adult cohorts. Gut. 2021;70(5):876-883. doi:10.1136/gutjnl-2020-320913
6. Ylonen, V., Lindfors, K., Repo M, et al. Non-biopsy serology-based diagnosis of celiac disease in adults is accurate with different commercial kits and pre-test probabilities. Nutrients. 2020:12(9):1736. doi:10.3390/nu12092736
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8. Wolf J, Petroff D, Richter T, et al. Validation of antibody-based strategies for diagnosis of pediatric celiac disease without biopsy. Gastroenterology. 2017;153(2):410-419.e17. doi:10.1053/j.gastro.2017.04.023
9. Ho SS, Keenan JI, Day AS. Role of serological tests in the diagnosis of coeliac disease in children in New Zealand. J Paediatr Child Health. 2020;56(12):1906-1911. doi:10.1111/jpc.15076

## Performance

### Method Description

*HLA-DQ* Typing:
LABType applies Luminex technology to the reverse sequence-specific oligonucleotide probe (SSO) DNA typing method. First, target DNA is polymerase chain reaction (PCR)-amplified using a group-specific primer. The PCR product is biotinylated, which allows it to be detected using R-Phycoerythrin-conjugated streptavidin. The PCR product is denatured and allowed to rehybridize to complementary DNA probes conjugated to fluorescently coded microspheres. A flow analyzer identifies the fluorescent intensity of phycoerythrin on each microsphere. The human leukocyte antigen (HLA) Class II allele or allele groups of the sample is determined by the positive and negative bead IDs using a computer software program. The assignment of the HLA typing is based on the reaction pattern compared to patterns associated with published HLA gene sequences.(Package insert: LABType SSO Typing. TDX-OLI-DMR-PS. One Lambda; Rev. 04, 11/2019)

Immunoglobulin A:
Total IgA levels are measured by immunonephelometry. In this Siemens Nephelometer II method, the light scattered onto the antigen-antibody complexes is measured. The intensity of the measured scattered light is proportional to the amount of antigen-antibody complexes in the sample under certain conditions. If the antibody volume is kept constant, the signal behaves proportionally to the antigen volume. A reference curve is generated by a standard with a known antigen content on which the scattered light signals of the samples can be evaluated and calculated as an antigen concentration. Antigen-antibody complexes are formed when a sample containing antigen and the corresponding antiserum are put into a cuvette. A light beam is generated with a light-emitting diode, which is transmitted through the cuvette. The light is scattered onto the immuno-complexes that are present. Antigen and antibody are mixed in the initial measurement, but no complex is formed yet. An antigen-antibody complex is formed in the final measurement. The result is calculated by subtracting the value of the final measurement from the value of the initial measurement. The distribution of intensity of the scattered light depends on the ratio of the particle size of the antigen-antibody complexes

to the radiated wavelength. (Instruction manual: Siemens Nephelometer II, Siemens, Inc.; Version 2.3, 2008; Addendum to the Instruction Manual 2.3, 08/2017)

Tissue Transglutaminase IgA/IgG Antibodies:
Immunoglobulin A and IgG antibodies to tissue transglutaminase (tTG) are detected by enzyme-linked immunosorbent assay (ELISA). Recombinant human tTG antigen expressed in *Escherichia coli* is adsorbed to wells of a microtiter plate under conditions that preserve the native state of the antigen. Diluted patient samples are added to the microtiter plate wells under conditions that allow binding of the antibodies to the tTG antigen. Unbound sample constituents are washed away, and horseradish peroxidase (HRP)-labeled antihuman IgA or IgG antibody conjugate is added to each well. After a second incubation, unbound HRP-label is removed, and bound conjugate is detected by adding tetramethylbenzidine (TMB) chromogenic substrate. After a final incubation, colored product is measured spectrophotometrically; the absorbance of the patient sample is compared to the positive calibrator. The absorbance is directly proportional to the level of IgA or IgG antibodies to tTG, which is expressed in arbitrary units.(Package inserts: QUANTA Lite R h-tTG IgA and IgG. Inova Diagnostics, Inc.; Rev. 8, 01/2020)

Deamidated Gliadin IgA/IgG Antibodies:
Immunoglobulin A and IgG antibodies to deamidated gliadin peptides are detected by ELISA. Purified peptides are adsorbed to wells of a microtiter plate under conditions that preserve the native state of the antigen. Diluted patient samples are added to the microtiter plate wells under conditions that allow binding of the antibodies to the deamidated gliadin peptides. Unbound sample constituents are washed away, and HRP-labeled antihuman IgA or IgG antibody conjugate is added to each well. After a second incubation, unbound HRP-label is removed, and bound conjugate is detected by adding TMB chromogenic substrate. After a final incubation, colored product is measured spectrophotometrically; the absorbance of the patient sample is compared to the positive calibrator. The absorbance is directly proportional to the level of IgA or IgG antibodies to deamidated gliadin peptides, which is expressed in arbitrary units.(Package inserts: QUANTA Lite Gliadin IgA II. INOVA Diagnostics, Inc.; Rev. 2, 10/2019; QUANTA Lite Gliadin IgG II. INOVA Diagnostics, Inc.; Rev.4, 05/2020)

### PDF Report
No

### Day(s) Performed
Profile tests: Monday through Friday; Reflex tests: Monday through Saturday

### Report Available
9 to 11 days

### Specimen Retention Time
Whole blood: 14 days; Extracted DNA: 2 months

### Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

## 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

See Individual Test IDs

## CPT Code Information

81376 x 2
82784 (if appropriate)
86258 (if appropriate)
86364 (if appropriate)
86231 (if appropriate)

## LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| CDGF | Celiac Disease Gluten-Free Cascade | 94493-4 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| DQA | DQ alpha 1 | 94495-9 |
| DQB | DQ beta 1 | 53938-7 |
| CELIG | Celiac gene pairs present? | 48767-8 |
| 28991 | Celiac Disease Interpretation | 69048-7 |