

Coeliac disease

Diagnosis, monitoring & susceptibility

Laboratory flowsheet included

'I have coeliac disease. What monitoring tests should be performed?'

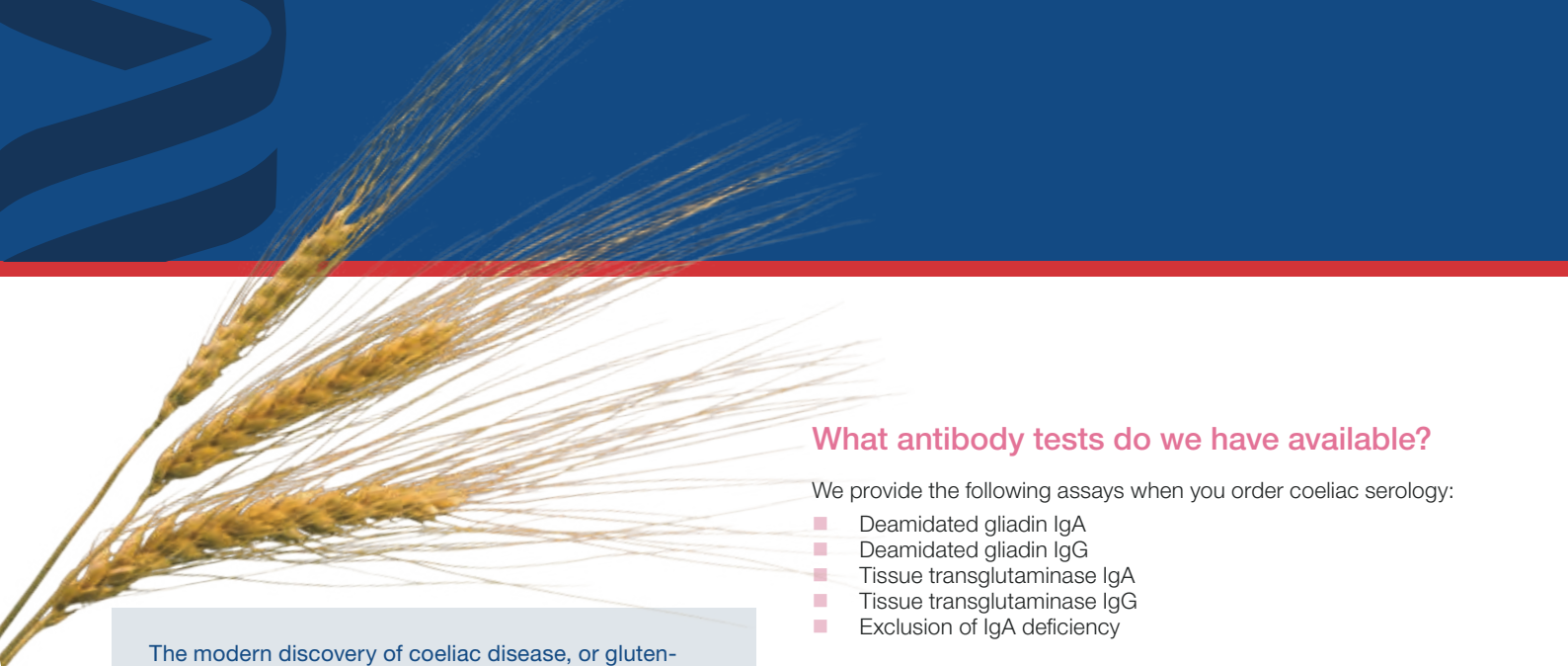
Do I have coeliac disease?

'Are either of our children susceptible to coeliac disease?'

Monitoring tests

Diagnostic tests

Genetic susceptibility tests



The modern discovery of coeliac disease, or gluten-sensitive enteropathy, followed wartime observations that some chronically unwell children improved with wheat deprivation and then deteriorated with improved food supplies, including wheat. The enteropathy is characterised by small intestinal lesions of variable severity which are provoked in genetically susceptible individuals by ingestion of gluten. Additional external trigger factors appear to be necessary. Almost all persons with coeliac disease have immune system genes (tissue types) coding for HLA-DQ2 or HLA-DQ8. The identification of these genotypes as necessary permissive factors for coeliac disease has helped the development of additional strategies in the investigation of patients who have, or are at risk of developing, coeliac disease.

A variety of autoantibody tests have been developed to facilitate the diagnosis of coeliac disease, which is confirmed by small bowel biopsy. Tissue transglutaminase (TTG) is the main, if not sole, autoantigen in coeliac disease. This enzyme is present in many different cell types, mainly in intracellular compartments. Tissue injury results in release of this enzyme where it has a role in tissue repair. The performance of tests measuring antibodies to TTG has been improved so that they have become the preferred screening and monitoring test over gliadin or endomysial antibody tests.

Enhancements to gliadin antibody tests have resulted in new tests with fewer false positives. Combining serologic markers can improve sensitivity and specificity. The purpose of this article is to put these advances into a clinical context, to assist you in the diagnosis and management of patients who may have coeliac disease.

1) Diagnosis

Doctor, do I have coeliac disease?

Coeliac disease has quite diverse manifestations and somewhat insidious features. It is not uncommon that patients may have a relative or friend in whom the diagnosis of coeliac disease and introduction of a gluten-free diet has transformed their life.

You should have some serology or antibody tests for coeliac disease.

What antibody tests do we have available?

We provide the following assays when you order coeliac serology:

- Deamidated gliadin IgA
- Deamidated gliadin IgG
- Tissue transglutaminase IgA
- Tissue transglutaminase IgG
- Exclusion of IgA deficiency

Our new Albia method

Our new assay detects IgG, IgA deamidated gliadin and tissue transglutaminase antibodies and also excludes IgA deficiency if present. This is done on the Bioplex 2200 as a multiplex Addressable Base Bead Immunoassay (ALBIA). This simultaneous assay excludes any risk of specimen integrity loss in testing. More markers mean more sensitivity and specificity.

IgA tissue transglutaminase antibody assays

This antibody is detected by ELISA tests and now with an ALBIA method. Although human TTG has been identified as 'the autoantigen' in coeliac disease, the slight differences of the TTG used in these assays may account for the observation that this assay does not achieve 100% sensitivity and specificity. The results exhibit a reasonable dynamic range, and the results are reported in units over a 'cut-off' value. Although this assay is not perfect, it has very high sensitivity and specificity. Some manufacturers have sought to enhance the performance of these assays by including peptides from gliadin, or gliadin in these assays. IgA antibodies to TTG become negative 9-24 months after commencement of a gluten-free diet. Like all IgA-based tests, the assay is useless in IgA-deficient individuals.

IgG tissue transglutaminase antibody assays

This assay has much less sensitivity for coeliac disease than all the other assays. Some patients with coeliac disease may be negative for TTG-IgG antibodies but have untreated coeliac disease. In general, most patients with IgA deficiency will have TTG-IgG antibodies.

Deamidated IgA and IgG gliadin antibody assays

IgA and IgG antibodies can be detected by ELISA tests and now with an ALBIA method. The results are reported in units over a 'cut-off'. The numbers or values of these results exhibit a good dynamic range of values. They mainly have utility for monitoring compliance with gluten-free diets in patients. Usually IgA gliadin antibodies are negative after 6-9 months of a gluten-free diet (normal <15 U/mL).

The IgG gliadin antibodies usually become negative 12-18 months after introduction of a gluten-free diet (normal <15 U/mL). This assay is very sensitive but less specific for identification of patients with coeliac disease. The results are almost invariably strongly positive in most patients with coeliac disease not on a gluten-free diet and low-level positives do not necessarily have clinical significance. An important advance has been made in deamidation of the gliadin antigen. This has reduced the number of false positives in IgG and now IgA gliadin antibody assays. Isolated gliadin IgA antibodies are usually false positives (when other markers are negative).

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IgA endomysial antibody assays

This antibody is detected by indirect immunofluorescence using monkey oesophagus as substrate. It is usually tested at one titre according to laboratory specific cut-offs (we use a titre of 1:10 but different laboratories' results may vary according to differences in tissue substrate and microscope).

This assay has very high specificity, but on occasion it can be less sensitive. With the current, new generation TTG tests, we believe this assay is useful only as a confirmatory assay for selected requests.

What are the common pitfalls in laboratory tests?

IgA-dependent serology is useless in patients with IgA deficiency, which occurs in 1:300 of the population, so measuring total IgA is a necessary part of the laboratory investigation.

Gluten restriction will result in lowering and subsequent disappearance of all of the lab markers.

Do not forget clinical symptoms from wheat exposure may result from other mechanisms, including IgE-mediated wheat allergy, non-immunological intolerance of wheat or preservatives used in wheat products (propionates).

Doctor, what do my tests mean?

1. I have been eating wheat and all the antibody assays are negative.

You do not have coeliac disease

2. I have been eating wheat and I have one or two low positive antibody tests.

You could have coeliac disease. You need not restrict wheat but should be monitored.

3. I have been eating wheat. Most of, or all of my four antibody markers are strongly positive.

You most likely have coeliac disease. This should be confirmed by endoscopy.

Doctor, do I need endoscopy and biopsy?

Patients with adequate gluten intake (at least four weeks of regular wheat) with abnormal coeliac markers should be referred for serology before biopsy, as negative serologic markers may raise the index of suspicion for other clinical problems.

Patients with unequivocal positive coeliac markers or IgA deficiency should be referred for endoscopy and biopsy to document the extent of mucosal abnormality and confirm the histopathology diagnosis.

2) Monitoring

I have coeliac disease. What tests should be performed?

Laboratory tests for the monitoring of coeliac disease

Genotyping studies are not useful for the monitoring of patients with known coeliac disease. IgA, IgG TTG antibodies and IgA, IgG gliadin antibodies should be requested in this setting.

My tests confirm coeliac disease. Are there any other tests that should be done?

Other useful tests might include estimations of iron studies, B12, vitamin D, IgG, IgA, IgM, full blood count, LFT, as well as a serum EPG. An important association of coeliac disease is type 1 diabetes. Some other autoimmune disorders are more common, such as pernicious anaemia, thyroid disease and vitiligo. Consider testing for these.

3) Susceptibility

Am I susceptible to coeliac disease?

1. I have not been eating wheat and don't think I could tolerate eating enough for a challenge.
2. I don't have symptoms but I want to know if I have a risk of coeliac disease because family members do.
3. My antibody tests have been discordant, some positive, some negative.

Possibly, you should have a coeliac tissue type test.

Tissue typing for HLA-DR and HLA-DQ is presently available through the laboratory and is reimbursed by Medicare. The detection of genes encoding HLA-DQ2 or HLA-DQ8 identifies individuals at risk of coeliac disease. The absence of these almost absolutely excludes coeliac disease, although more people have an 'at risk' genotype than those who will get the disease. Genotype results are not affected by wheat consumption in the diet and are usually performed on a dedicated EDTA specimen.

The likely clinical utility of these tests is that they will be useful for identifying individuals from families known to have coeliac disease who will need longer term surveillance. We report both the phenotype and the genotype, as well as homozygosity and heterozygosity.

The risk status of persons with indeterminate or equivocal serology, discordant serology and biopsy results or inadequate dietary intake of gluten can also be established.

A particularly attractive feature of genotyping is that gently scraping the buccal mucosa with an appropriate collection device can provide sufficient DNA for testing young children, obviating the need for venepuncture.

Increased risk determined by "coeliac genotyping"

Haplotype	Risk
DQ2, B1*02/*02	1:7
DQ2 and DQ8	1:41
DQ8, B1*02 or B1*0302 pos	1:43
B2, B1*02/*02	1:45
DQ2, B1*02/X ¹	1:47
B2, B1*02/X ¹	1:75
DQ8, B1*02 neg	1:85

X¹ = any other type

from B.Piccini (et al) REV ESP ENFERM DIG 2012;104 (5):254

Are the lab tests going to be helpful if I am going to send the patient for an endoscopy anyway?

If the laboratory tests for coeliac disease are negative, you will not have to refer a patient for an endoscopy to diagnose coeliac disease. Endoscopy may still be appropriate when clinical entities other than coeliac disease may be considered. Not all patients may need to have follow up endoscopies if they have a good clinical response to gluten restriction and demonstrate normalisation of laboratory markers of coeliac disease (gliadin, endomysial or tissue-transglutaminase antibodies) and its consequences (iron deficiency, changes in blood count at presentation). For most patients, a progress endoscopy after introduction of gluten restriction is not unreasonable.

The patient said “I stopped eating wheat a long time ago, and now I want to know if I have coeliac disease.” What can I advise?

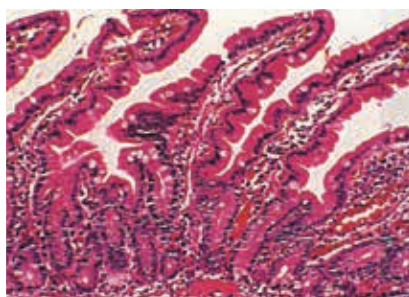
This is perhaps the most difficult situation in that such patients may be very reluctant to eat wheat again.

For the diagnosis of coeliac disease, endoscopy is not likely to be rewarding if the laboratory markers are negative.

When patients have avoided wheat products for a long period of time, coeliac markers are often negative or indeterminate. It is reasonable to check the laboratory markers and negotiate a period of wheat ingestion with repeat laboratory tests and consideration of referral for endoscopy. Coeliac HLA-DR/DQ genotyping can be used to include or exclude patients in this setting.

If I have to persuade the patient to eat wheat again for the serology and biopsy, how long do they have to do this for?

It is difficult to provide clear evidence-based advice on this but a minimum of one serve a day for four weeks would be a reasonable “rechallenge” period.

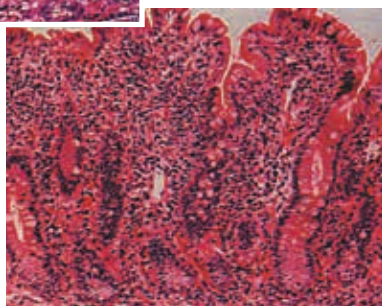


Normal duodenal mucosa

◀ Note the long “finger-like” villi on the surface.

Duodenal mucosa in coeliac disease

The surface is almost flat, showing sub-total villous atrophy. These mucosal changes can revert to normal or near normal on a gluten-free diet. ▶



We have done all the coeliac tests and they are negative. The patient asks “OK, so I don’t have coeliac disease but why do I feel better if I avoid wheat?” What do I say?

IgE-mediated wheat allergy

Some people have IgE-mediated allergy to wheat, other cereal grain proteins, legumes (soy, lupins), or even seeds, such as sesame or poppy seeds, which they might encounter in bakery products. Allergens in these foods are often more concentrated in the seeds or grains. These symptoms may be tested by RAST or skin prick tests as well as avoidance and challenge.

Wheat intolerance

Wheat intolerances result in clinical symptoms, commonly ‘irritable bowel symptoms’, and sometimes in less specific symptoms that can result from wheat ingestion, be alleviated by wheat avoidance, and recur with wheat challenge.

Fructose intolerance

Some people are fructose deficient and get irritable bowel syndrome with high fructose foods, including wheat.

Preservative intolerance

Many bakery products also contain preservatives (most commonly propionic acid) and this can result in a broad range of symptoms in some persons, including angioedema, urticaria, irritable bowel symptoms, as well as headaches, ‘fuzzy heads’ and fatigue. There are no laboratory tests for these ‘intolerances’, which are best characterised by dietary restriction and challenge.

Diagnosis of coeliac disease

Currently symptomatic	Coeliac serology (TTG, gliadin Abs)
Monitoring	TTG antibodies, gliadin antibodies. Also iron studies, Vitamin B12 and D, IgG, IgA, IgM, full blood count, LFT. Serum EPG may be considered initially.
Asymptomatic child of coeliac family Predisposition to coeliac disease	Coeliac tissue typing on buccal smear or EDTA blood
Possible inadequate wheat intake	Coeliac tissue typing and coeliac serology (TTG, gliadin Abs)
Discordant serology*	Coeliac tissue typing, endomysial IgA, repeat TTG gliadin Abs, Total IgA

*Most low level positives (<30 IU/mL) or isolated positives are false positives. Patients with discordant results need not restrict wheat but coeliac serology should be monitored periodically.

If you have any enquiries regarding coeliac disease, please contact Dr Michael Metz on (08) 8366 2000