NATIONAL LABORATORY HANDBOOK

Serological Testing for Coeliac Disease

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National Clinical & Integrated Care Programmes
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PATHOLOGY

ROYAL COLLEGE OF PHYSICIANS OF IRELAND

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Scope

The aim of this guideline is to provide indications and guidance for serological testing for coeliac disease, including circumstances where testing is not required, or when retesting may be indicated. These guidelines can be used by clinicians and clinical laboratories, and apply to children & adults.

Key Recommendations for Clinical Users

Laboratory testing for coeliac disease should be reserved for specific patient groups with indications for testing as described below:

- Gastrointestinal /extraintestinal manifestations of malabsorption,
- First degree relatives of patients with coeliac disease,
- Osteoporosis; unexplained neurological conditions,
- Unexplained abnormal LFTs,
- In symptomatic paediatric patients – gastrointestinal and non-gastroentestinal,
- In asymptomatic paediatric patients with an associated condition such as T1 diabetes mellitus, Down syndrome, selective IgA deficiency or autoimmune thyroiditis.

Note: one in thirty people with coeliac disease is IgA deficient, and therefore negative IgA based serology does not exclude coeliac disease, unless IgA deficiency is also excluded.

Dietary advice should be given to patients prior to undergoing investigations for coeliac disease:

- explain that testing is accurate only if a gluten-containing diet is eaten during the diagnostic process **and**
- advise the patient not to start a gluten-free diet until diagnosis is confirmed by a specialist, even if the results of a serological test are positive. [1,2]

Advise people who are following a normal diet (containing gluten) to eat some gluten in more than 1 meal every day for at least 6 weeks before testing.

If people who have restricted their gluten intake or excluded gluten from their diet are reluctant or unable to re-introduce gluten into their diet before testing:

- refer the person to a gastroenterologist **and**
- explain that it may be difficult to confirm their diagnosis by intestinal biopsy.[1]

Key Recommendations for Laboratories

- Measurement of IgA anti-tTG antibodies (tTG) is the appropriate screening test for coeliac disease.
- Equivocal or positive sera should be reflex tested for IgA anti-Endomysial antibodies (EMA).
- In cases with a negative tTG result, IgA deficiency should be excluded.
- If total IgA level is low, IgG EMA or other IgG-based serological testing should be performed.

All serological tests should be validated & accredited to ISO15189 as inspected by the Irish National Accreditation Board (INAB).

Background & Epidemiology

Coeliac disease (CD) is an autoimmune disease in which ingestion of gluten causes chronic inflammation of the small intestine, which can lead to malabsorption of nutrients. CD has a multifactorial aetiology with strong HLA linkage. HLA-DQ2.5 is found in some 95% of patients with coeliac disease. This molecule is composed of an alpha chain and beta chain. The allele coding for the alpha chain is HLA-DQA1*0501 and the allele coding for the beta chain is HLA-DQB1*0201. [3]

The prevalence of CD in Europe and the US has been increasing, with attempts at disease prevention aimed at modifying weaning practices around infant feeding. Previous guidance by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) concerning introduction of gluten had recently been revised, with gluten introduction now recommended between 4 and 12 months of age. Although there are other health benefits, there is no evidence that breast feeding is protective against development of CD. In children at high risk of CD, early introduction of gluten is associated with earlier onset of CD; however the cumulative incidence later in childhood is similar. There is observational evidence that ingestion of large amounts of gluten during weaning is associated with an increased risk of CD; however, the optimal amount of gluten during weaning and infancy remains to be defined. [4]

CD can cause gastrointestinal symptoms of malabsortion and extra-intestinal manifestations (anaemia, vitamin deficiencies, weight loss/growth failure, fatigue and stomatitis). National Institute for Health and Care Excellence (NICE) guidelines recommend testing symptomatic patients, as well as first degree relatives of those with coeliac disease, patients with Type 1 diabetes, autoimmune thyroid disease, osteoporosis, unexplained neurological symptoms, unexplained subfertility / miscarriages, unexplained abnormal liver function tests, dental enamel defects, Trisomy 21 and Turner syndrome.[1]

CD is estimated to affect 1% of the Irish population, and the incidence is rising worldwide. Clinical improvement is achieved by adherence to a lifelong gluten-free diet as the main treatment. Complications of coeliac disease, which may or may not be present at diagnosis include osteoporosis, ulcerative jejunitis, malignancy (including lymphoma), functional hyposplenism and nutrient deficiencies.[5] Many patients' symptoms improve rapidly on

commencing a gluten free diet; however, some patients can remain symptomatic for several months. In patients who are slow to respond to dietary management, inadvertent gluten ingestion should be excluded.

Approximately 115,000 coeliac screens are performed annually in Ireland, accounting for 0.15% of all blood science tests. Of these, follow up endomysial antibody testing is required in approximately 4,140 samples. Some of these, 676 (16%) require IgG based endomysial antibody testing or other IgG based serology, because of a low or undetectable total IgA level.

IgA deficiency is present in about 1:30 patients with coeliac disease (and about 1:600 of the general population). When IgA deficiency is present IgA tTG testing can give false negative results. Thus in patients with IgA deficiency an IgG anti-EMA test (or other IgG serological testing) should be performed which if strongly positive is suggestive of coeliac disease.[5] Additionally IgA deficiency & other humoral immunodeficiencies can mimic coeliac disease, as patients may present with gastrointestinal symptoms such as chronic diarrhoea, malabsorption or abdominal pain due to either autoimmune complications or chronic infections.

If a low serum IgA is detected, immunoglobulins & serum protein electrophoresis (SPEP) should be performed to exclude a more extensive hypogammaglobulinaemia.

Patients on a gluten free diet will have negative serology and the biopsy will normalise.

Testing

Testing for IgA anti-tTG antibodies is used to identify patients most likely to have coeliac disease. Effectively serology screens patients to identify those who need further diagnostic evaluation by endoscopy. Anti-tTG antibody measurement can aid in monitoring adherence to a gluten-free diet.

Who to Test

Testing for coeliac disease **is recommended** in the following clinical circumstances:

- persistent unexplained abdominal or gastrointestinal symptoms,
- faltering growth,
- prolonged fatigue,
- unexpected weight loss,
- severe or persistent mouth ulcers,
- unexplained iron, vitamin B12 or folate deficiency,
- type 1 diabetes mellitus, at the time of diagnosis,
- autoimmune thyroid disease, at the time of diagnosis,
- irritable bowel syndrome (in adults),
- first-degree relatives of people with coeliac disease.[1,2]

It is important to remember that patients may be overweight or obese, and an elevated body mass index (BMI) does not exclude the diagnosis.

Paediatric approach to coeliac diagnosis

The clinical indications to test paediatric patients for coeliac disease are outlined above. In children, a total IgA level plus IgA anti-tTG antibodies are recommended at the time of testing. Patients with abnormal results should be referred to a paediatric gastroenterologist for further assessment, in line with current ESPGHAN guidelines. [6] These guidelines were somewhat controversial as they were not validated prior to publication, and revised guidelines from ESPGHAN are expected later in 2019. Biopsy-based diagnosis remains the reference

standard for diagnosis of children with coeliac disease. It is a routine and safe procedure when conducted in experienced, specialist paediatric facilities. ESPGHAN guidelines offer gastroenterologists a non-biopsy option for making a diagnosis in those with IgA-tTG levels above 10 times the upper limit of normal and a separately positive anti-EMA antibody. Testing all potential patients for the HLA risk alleles is of questionable merit.[7] Patients detected through routine screening (e.g. first degree relatives, Down syndrome, diabetes mellitus etc) require diagnostic biopsy analysis.

Who Not to Test

Testing for anti-tTG is **not recommended** in the following circumstances:

- Do not include in "Routine Bloods", health-screening requests, or other forms of screening.
- Do not offer serological testing for coeliac disease in infants before gluten has been introduced into the diet.
- Do not test patients on a gluten free diet unless monitoring response to treatment in a known coeliac patient.
- Healthy children with appropriate weight trajectory and growth velocity, unless they meet specific screening criteria as discussed above.[6,8]

A meta-analysis found little or no evidence of the benefits or the harm caused by screening for coeliac disease in asymptomatic individuals. More research is needed to understand the effectiveness of screening and treatment for coeliac disease, accuracy of screening tests in asymptomatic persons, and optimal screening strategies. [9]

Who to Re-Test

- IgA anti-tTG can be used to monitor response to a gluten free diet. Retesting may be performed at 6–12 months depending on pre-treatment value.[10]
- A negative anti-tTG indicates that coeliac disease is unlikely if the patient is on a normal gluten-containing diet. If clinical suspicion is high, testing should be repeated after 3-6 months, ensuring that the patient is on a diet with a normal gluten content.
- If a retest sample in a patient on a gluten containing diet is normal, alternative causes for symptoms should be sought.

Specimen and Ordering Information

All requests (electronic & paper) & specimens must adhere to the laboratories standard requirements. In order to comply with accreditation standards, laboratories cannot accept or process samples which do not meet minimum standards.

Testing for coeliac disease is only accurate if the patient has been adhering to a gluten-containing diet for at least 6 weeks, and preferably 3 months (i.e. gluten in one or more meals/day, min 15g gluten/day).

No special specimen precautions are required.

How to Test

All serological tests should validated & accredited to ISO15189 as inspected by the Irish National Accreditation Board (INAB).

Laboratory testing for suspected coeliac disease should include the following:

- Measurement of IgA anti-tTG antibodies (tTG).
- Samples found equivocal, weak positive or positive for anti-tTG should have a confirmatory EMA test performed. This test should be reflexed within the laboratory

and if not available on site should be sent to an accredited lab that performs EMA testing.

- Exclude IgA deficiency (by measurement of total IgA or other validated methods).
- In cases of IgA deficiency, IgG EMA testing or other IgG serological testing is performed.
- Sequential testing offers optimal diagnostic utility.

Laboratories should ensure both results & their interpretation are clearly communicated to healthcare professionals.

Interpretation of Tests

Anti-tTG has a high sensitivity for untreated coeliac disease, while the anti-Endomysial antibody is more specific.

Result	Interpretation
Negative IgA anti-tTG & IgA deficiency excluded	Coeliac disease unlikely if the patient is on a normal diet. If clinical suspicion is high, anti-tTG should be repeated in 3-6 months, ensuring that the patient is on a diet with a normal gluten content.
Equivocal IgA anti-tTG	All equivocal results should be further tested for IgA anti-EMA.
Positive IgA anti-tTG	Suggestive of coeliac disease. All samples with positive anti-tTG should be further tested for anti-EMA antibodies by indirect immunofluorescence.
Negative IgA anti-EMA, with positive anti-tTG	Coeliac disease is unlikely if patient is on a normal diet. However false negative results may be seen in IgA deficiency, and also in patients on a gluten free diet. The clinical significance of a negative EMA in a patient with a positive anti-tTG is uncertain; however an expert GI opinion should be sought in this situation, as biopsy may still be indicated.
Positive IgA anti-EMA	Suggestive of coeliac disease.
Negative IgA anti-EMA & low total IgA	In this setting, negative anti-EMA does not exclude coeliac disease. IgG based serology should be performed.
Negative IgA & IgG anti-EMA & low total IgA	The negative predictive value of serology in this setting is less than in those with normal IgA. If there is a strong clinical suspicion of coeliac disease, suggest referral to a consultant gastroenterologist.
Low total IgA	Request immunoglobulins & SPEP to exclude a more extensive hypogammaglobulinaemia. However patients with isolated IgA deficiency are at risk of infections, allergy, autoimmune disease & serious transfusion reactions. If total IgA is undetectable, referral to a clinical immunologist may be considered.

Recommendations for National Laboratory Information System (MedLIS)

- Anti-Endomysial antibodies (IgA), anti-Endomysial antibodies (IgG) & other IgG based serology should be Lab Orderable Only.
- When there is a high clinical suspicion of coeliac disease, but the anti-tTG is negative, clinicians should be encouraged to contact the laboratory, and laboratories should review results and provide an anti-endomysial antibody for confirmation. These requests for additional testing should be reviewed, and used as part of validation of cut-off for the anti-tTG assay.
- Coeliac Screen should be included as test type/searchable term and should map to an anti-tTG, and measurement of IgA / exclusion of IgA deficiency by background analysis +/- direct measurement.
- Retest interval of 6 months for a known coeliac.
- A 2nd test on a patient for diagnosis should be allowed after 8 -12 weeks.
- Following negative testing for a patient eating a gluten containing diet, further repeat testing is not indicated for >12 months.(10)

Information for Patients

Coeliac disease is an autoimmune disease where gluten in the diet, activates the patient's own immune system. Gluten is a protein found in wheat & other cereal grains. The immune system's reaction in the intestinal wall gradually flattens the finger-like projections (villi) lining the gut surface that absorb nutrients from food.(4) Symptoms of coeliac disease include bloating, diarrhoea, weight loss & abdominal pain caused by inflammation of the gut.

The reference standard for diagnosis is small bowel mucosal biopsy which involves an endoscopic procedure to take a small piece of tissue from the intestine. The tissue sample is stained and the structure examined under the microscope. (11) Pathologists look for signs of inflammation and damage to the intestinal lining.

The first step is usually a blood test to detect specific autoantibodies known as anti-tissue transglutaminase antibodies (tTG) which are present in most people with coeliac disease, who are eating gluten in the diet. If anti-tTG antibodies are present, the lab will add on a confirmatory anti-endomysial antibody (EMA) test. The anti-endomysial test is rarely positive in people who do not have coeliac disease, and so the combination of the 2 tests will give your doctor the best information possible from a blood test.

Testing for coeliac disease is only accurate if you have been regularly eating a gluten-containing diet i.e. gluten in one or more meals/day for at least 6 weeks.

If you have a positive coeliac blood test (tTG/EMA), you will be referred to a specialist & advised to continue with a gluten containing diet until your diagnosis is confirmed.

There is excellent information available from the Coeliac Society of Ireland. (www.coeliac.ie) (12)

Guideline Development Methodology - Consultation Plan and History

The guideline was drafted by the authors, following which expert consultation with the Irish Society for Gastroenterology and the Irish Academy for Allergy and Immunology was undertaken. Following incorporation of feedback, the guideline was submitted to the full National Clinical Programme for Pathology Consultation Process.

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