

Pernicious anaemia was first described over 150 years ago by Thomas Addison. Since then, much has improved in terms of diagnosis and treatment. Here, Philip Day provides an overview of this most common cause of vitamin B₁₂ deficiency and describes the value to patients of an automated intrinsic factor antibody test.

Intrinsic factor antibody testing

Automated diagnosis of pernicious anaemia

Pernicious anaemia (PA) is the most common cause of vitamin B₁₂ deficiency. It is caused by an autoimmune gastritis that affects the gastric parietal cells, leading to the destruction of the gastric mucosa, reduced or absent gastric acid production and a lack of intrinsic factor (IF). The condition is characterised by vitamin B₁₂ deficiency, megaloblastic anaemia, neuropathy and gastritis with IF autoantibodies. Pernicious anaemia affects around one in a 1000 people in northern Europe, and tends to run in families. It is very uncommon in children and the incidence increases with age.

Two autoimmune processes lead to PA. First, there is depletion of IF-producing gastric parietal cells, and then patients produce autoantibodies that block the binding sites on the IF required for B₁₂ absorption. When B₁₂ deficiency is identified, vitamin replacement therapy should commence immediately, even if further investigations are required, because the patient's condition will continue to deteriorate.

The response to simple B₁₂ therapy is very rapid: a reticulocyte response is normally seen within 48 to 72 hours. However, changes to bone-marrow morphology immediately after treatment can be misleading.

History of pernicious anaemia

Pernicious anaemia was first described in detail by Thomas Addison in 1849. Almost 60 years later, in 1907, Richard Clarke Cabot reported on a series of 1200 patients with PA and quantified their average survival to between one and three years. Early treatment

‘Early treatment for pernicious anaemia was pioneered by George Minot and William Murphy, who fed patients a diet rich in lightly cooked liver’

for PA was pioneered by George R Minot and William P Murphy in 1925. They fed patients with a diet rich in ‘lightly cooked liver’ and found that they improved.¹ Liver is a rich source of vitamin B₁₂ but does not contain intrinsic factor (IF), which is produced in the stomach.²

Despite a cure being available for PA, William Bosworth Castle undertook a study in which he demonstrated the positive effects of feeding PA patients with meat treated with gastric juice, which contains intrinsic factor. He fed one group of 10 patients raw beef and another group raw beef treated with normal gastric juice, which he prepared by ingesting raw hamburger meat himself and regurgitating it after an hour. The patients fed regurgitated beef showed a reticulocyte response, whereas no response was observed in the other group.

Intrinsic factor and autoantibodies

Intrinsic factor is a glycoprotein produced by the gastric parietal cells. It binds to, transports and facilitates absorption from the terminal ileum of the very small amount of vitamin B₁₂ in the diet. If there are antibodies to either the parietal cells, the B₁₂ binding

site of IF or the binding site of IF to the ileum, the patient's ability to absorb dietary B₁₂ by the IF route will be reduced.

Over time, the presence of these antibodies leads to a reduction in B₁₂ stores and ultimately to B₁₂ deficiency, the consequences of which vary. The classical haematological manifestation is a macrocytic anaemia due to megaloblastic changes in the bone marrow, although this is not always a presenting feature because any co-existing iron deficiency will mask the macrocytic changes to some degree. Some patients first present with diverse neurological symptoms, ranging from peripheral neuropathy to confusion and memory loss.

The presence of circulating autoantibodies to IF is a very specific indicator of pernicious anaemia. Antibodies against intrinsic factor (IFAb) are found in approximately 50% of cases but rarely in other conditions. Antibodies to gastric parietal cells are found in 90% of cases of PA. However, this apparent greater sensitivity is offset by a lack of specificity because antibodies directed at parietal cells are found in a range of other conditions, the incidence of which increase with age.

Intrinsic factor has two binding sites: the B₁₂ binding site and the site whereby the IF complex binds to the ileal receptor. The most common IFAb (type I) is directed at the B₁₂ binding site of IF; the less common type II IFAb appears to be directed against the ileal receptor binding site. Either antibody will interfere with IF-mediated B₁₂ absorption. It is vital that any test detects the more common type I antibodies. Type II antibodies are normally associated with the type I variety, and it is unclear whether or not type II antibodies ever occur in isolation.

Automation reduces turnaround time

Tests of variable sensitivity and specificity have been performed for IFAb for many years. Early in-house tests utilised radiolabelled B₁₂ and were very laborious. Later commercial kits simplified and streamlined the laboratory process but the tests were still batched and turnaround time remained dependent on laboratory workload and staffing levels.

Recently, a rapid, random-access automated test for IFAb has been released by Beckman Coulter for use on its Access and UniCel DxI 800 immunoassay analysers to complement the serum B₁₂ assay. It is very simple to set up, uses single-point calibration (stable for 14 days) and produces the first result in 36 minutes from sampling. Results are then produced at the rate of around one per minute. It is simpler and carries

‘The glycoprotein intrinsic factor binds to, transports and facilitates absorption from the terminal ileum of the small amount of vitamin B₁₂ in the diet’

less risk of sample loss or deterioration than batch tests (Fig 1).

While IFAb results may not be required urgently on medical grounds, it is a quick and easy test to administer compared with the Schilling test – the traditional method of assessing B₁₂ absorption. In comparison, the Schilling test can be stressful and sometimes unpleasant for the patient, is expensive for the hospital and always time-consuming.

Performing the IFAb test alongside the serum B₁₂ simplifies reflex testing, where this is allowed. A low serum B₁₂ level and an IFAb result together provide the clinician with more useful information than serum B₁₂ level alone. From the patient’s point of view,

this can represent a significant advantage.

When a patient with a low serum B₁₂ level is shown to have IFAbs it indicates that the B₁₂ deficiency is due to difficulties in absorption and transportation – a diagnosis of pernicious anaemia. In these cases it eliminates the need for the Schilling test. If the IFAb test is not performed and there is a delay in deciding to run the Schilling test, PA may go undiagnosed and the patient will run a far higher risk of developing one of the serious complications associated with B₁₂ deficiency.³

Schilling test

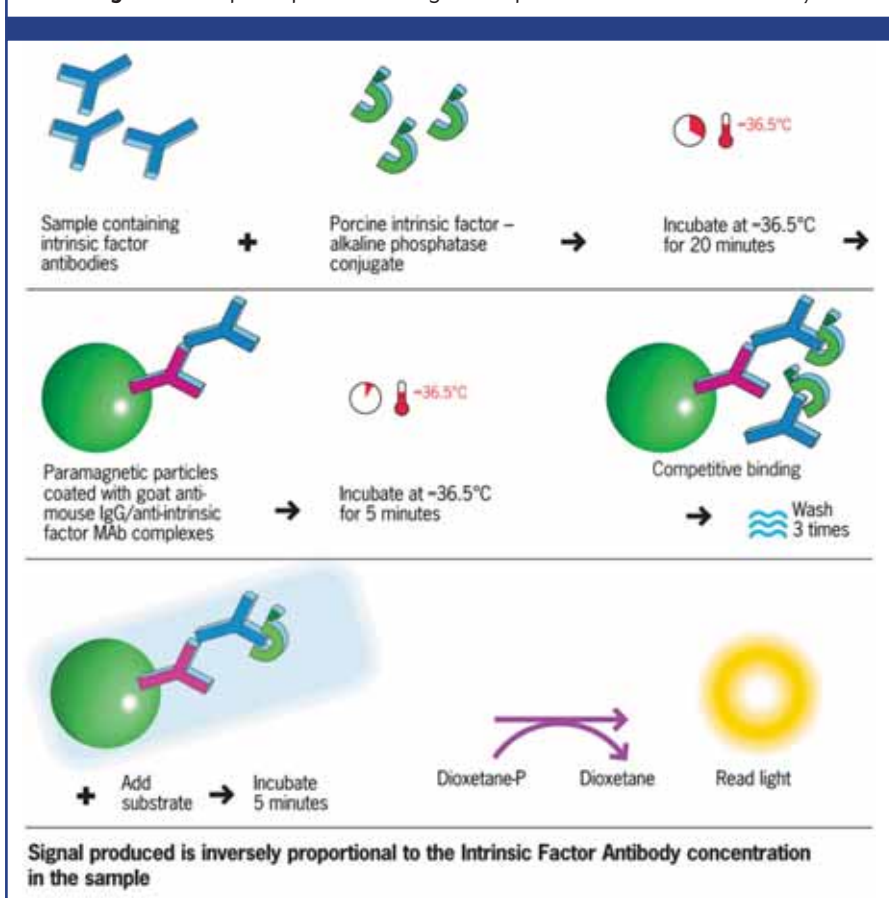
The traditional method for assessing B₁₂ absorption has been the Schilling test. This is an expensive test, with one dose of the radioactive B₁₂ required for the test costing £112 in the UK in 2005. It is also laborious and cannot be conducted immediately. A patient presenting with B₁₂ deficiency has to wait several months for the test to be performed because it should not be undertaken while there is B₁₂ deficiency. The deficiency is best corrected initially, otherwise it may affect the mucosal lining of the stomach and intestine, impairing vitamin B₁₂ absorption simply as a result of megaloblastosis-associated malabsorption. This is particularly relevant in severe deficiencies of B₁₂ and/or folate.

The Schilling test requires the patient to receive an intramuscular injection of non-radioactive vitamin B₁₂ and an oral dose of radiolabelled B₁₂. All urine passed in the following 24 hours is collected and the radioactivity is measured. Vitamin B₁₂ absorbed from the intestine into the bloodstream is excreted in the urine as it cannot be utilised because the binding proteins are saturated by the intramuscular dose. The remainder of the oral dose is excreted in faeces.

In order for the test to be valid, the patient must have adequate renal function and comply with the 24-hour urine collection. If renal function is impaired, the excretion of the isotope may be compromised.

If an abnormally low result is obtained, the process may be repeated one month later with the addition of IF to the oral dose

Fig 1. Two-step competitive binding technique for intrinsic factor antibody.



‘Intrinsic factor antibody tests are more cost-effective and less laborious than the Schilling test and are simpler for the laboratory to perform’

Table 1. The patient's viewpoint: three ways to diagnosis.

TIME (WEEKS)	NO IFAB TEST	IFAB TEST, BUT REFERRED TO OTHER HOSPITAL	AUTOMATED IFAB ALONGSIDE HAEMATINICS TESTS
0	Visit GP: FBC and haematinics samples taken	Visit GP, FBC and haematinics samples taken	Visit GP: FBC and haematinics samples taken. IFab reflexed if B ₁₂ low
2	Visit GP: receive results, B ₁₂ prescribed and hospital referral arranged	Visit GP: receive results, B ₁₂ prescribed, IFab sample taken	Visit GP: receive results and interpretation, B ₁₂ prescribed. Refer if desired
3	Visit nurse, start B ₁₂ injections	Visit nurse: start B ₁₂ injections	
4-6	Waiting time	Waiting time	
7		Visit GP: receive IFab result, diagnosis and/or refer	
20	Visit clinic: Schilling test ordered		
24	Visit laboratory: undergo Schilling test, take away large container		
25	Visit laboratory: return container of urine		
26	Visit clinic: receive results and possibly a diagnosis, or further investigations booked (eg part II Schilling test)		

(part II Schilling test). If increased absorption is seen with IF, it is assumed that the IF has facilitated the absorption, thereby supporting the diagnosis of PA. If there is no improvement in absorption, it is assumed that the vitamin B₁₂ deficiency is due to malabsorption.

The Schilling test is a functional assay that can demonstrate the ability of the intestine to absorb B₁₂, but it does not guarantee that dietary vitamin B₁₂ will be absorbed to the same degree.

Value of earlier treatment

Although vitamin B₁₂ deficiency can be treated simply with regular vitamin B₁₂ replacement therapy, and this should be undertaken as quickly as possible, it is important to establish a diagnosis of PA. This is because it is an autoimmune condition and patients with PA have a higher incidence of other autoimmune syndromes and also a higher incidence of gastric cancer. Pernicious anaemia is not a 'diagnose, treat and forget' condition.

It is reasonable to test all samples for IFab when serum B₁₂ levels are low, as well as samples where IFab levels are requested specifically, regardless of B₁₂ level, when the patient has symptoms that may be associated with B₁₂ deficiency. Vitamin B₁₂ deficiency cannot be excluded when the serum level is below 400 ng/L.⁴

When all patients with low serum B₁₂ samples are tested for IFab, a large proportion will show a positive result, indicating PA. However, not all cases will be detected and some patients who previously

have had low B₁₂ levels will have been repeat tested. The Schilling test should only be considered for patients who test negative for IFab, once their serum B₁₂ level has been in the normal range for several weeks. It should be reserved for these patients because it is expensive and laborious and requires patients to wait much longer for their results (Table 1).

Earlier batched IFab tests did not completely replace the Schilling test and automation of the IFab test will not do this either. However, the availability of a rapid IFab test should mean that the Schilling test is requested less frequently, and only for patients who have tested negative for IFab.

Intrinsic factor antibody tests are more cost-effective and less laborious than the Schilling test and are simpler for the laboratory to perform. Automation of IFab

'Pernicious anaemia is an autoimmune condition and affected patients have a higher incidence of other autoimmune syndromes and of gastric cancer'

detection will speed up the time to result even further for the patient. The benefit to the patient is considerable, reducing the time to diagnosis of PA for those patients who are IFab-positive.

In some instances patients with B₁₂ deficiency are treated but a diagnosis of PA is never established through assessment of IFab or the Schilling test. Therefore, such patients are unaware of the increased risks of other autoimmune syndromes and gastric cancer. Automated IFab is an inexpensive and simple way of identifying this increased risk for 50% of patients with pernicious anaemia. ■

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