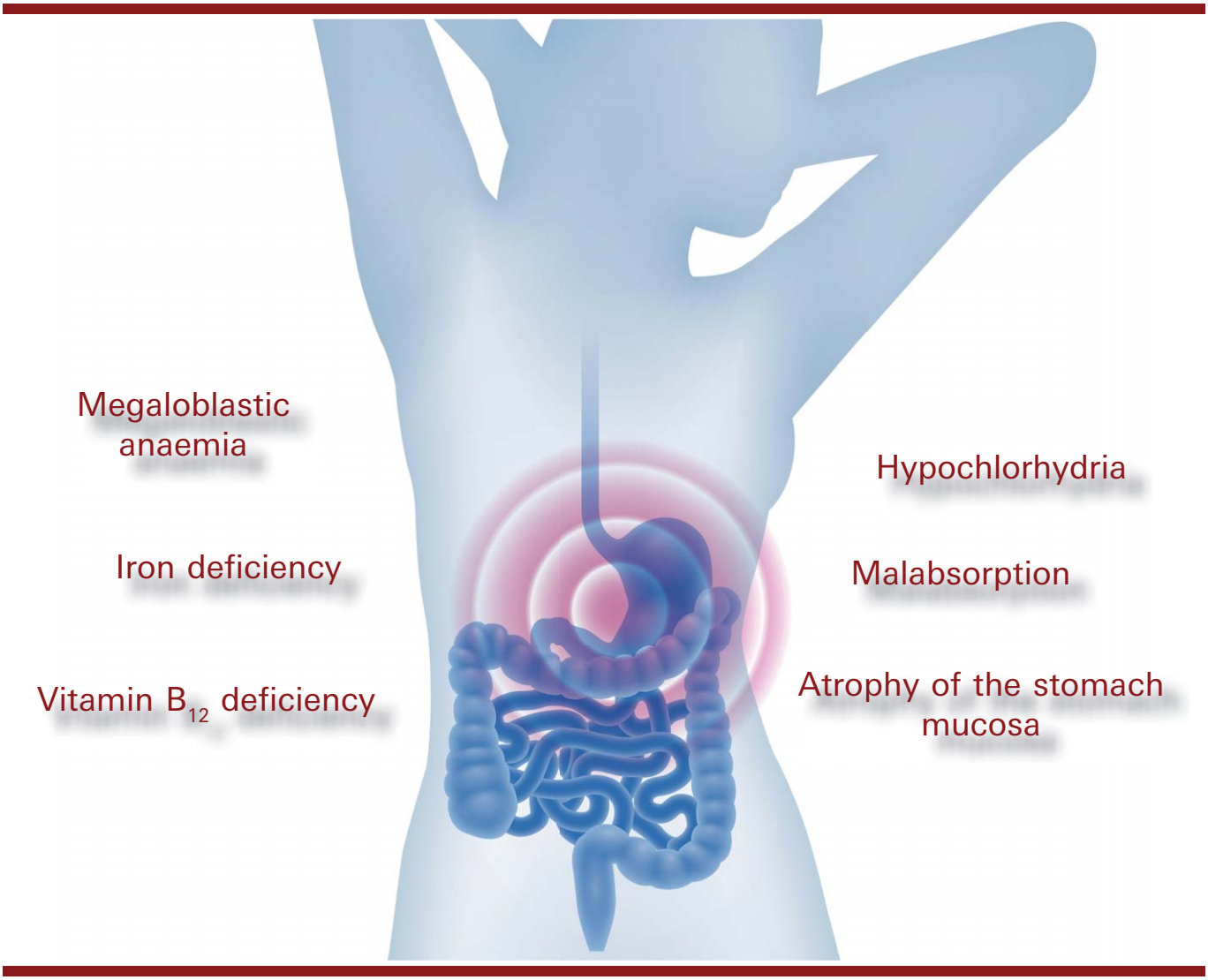
EUROIMMUN Medizinische Labordiagnostika AG

Autoimmune gastritis and pernicious anaemia

Differential diagnostics for an insidious disease

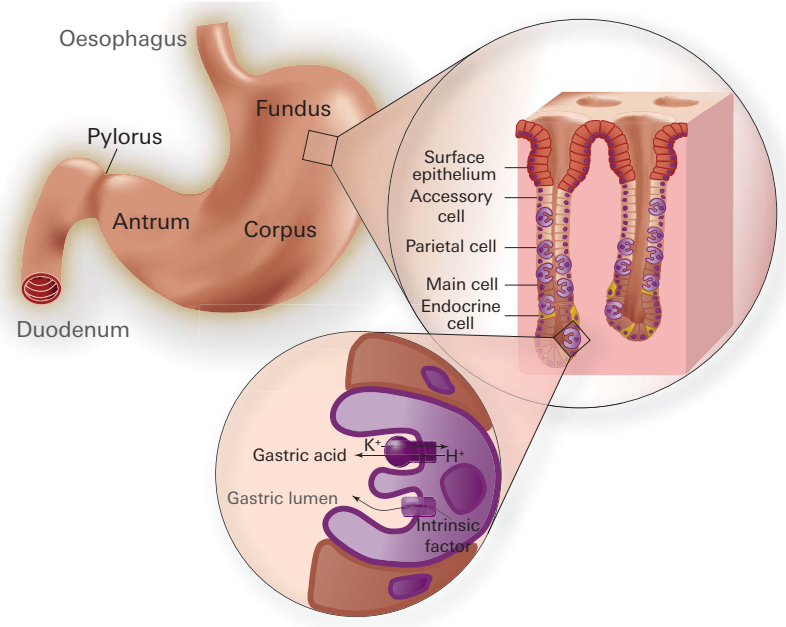


- **IIFT: Stomach (Monkey), Anti-Intrinsic Factor IIFT and EUROPLUS Stomach (Monkey)/ Intrinsic Factor**
  - Specific detection of antibodies against PCA and intrinsic factor (IF)
- **Anti-PCA ELISA (IgG), Anti-ATP4B ELISA (IgG) and Anti-Intrinsic Factor ELISA (IgG)**
  - Reliable confirmation and quantification of antibodies against PCA and IF
- **EUROLINE Autoimmune Gastrointestinal Diseases (IgG)**
  - Discrimination of autoimmune gastritis from Crohn's disease and coeliac disease

Autoimmune gastritis and its consequences

Autoimmune gastritis (AIG) is a chronic inflammation of the mucous membranes in the fundus and corpus of the stomach. Immune cells attack the oxyntic mucosa, resulting in the destruction of the parietal cells. AIG with an asymptomatic course for many years can develop into chronic atrophic gastritis, which may manifest as iron deficiency anaemia or pernicious anaemia (PA).[1,2]

The cause of AIG is unknown. The disease is characterised by autoantibodies against parietal cells (APCA) of the stomach mucosa and against intrinsic factor (IF).[3] The primary target antigen of APCA is the β-subunit of H+/K+-ATPase (ATP4B), a proton-potassium pump, which is required for the production of gastric acid. Intrinsic factor is a glycoprotein that is secreted by parietal cells. It binds vitamin B_{12} and thus enables its absorption into the terminal small intestine.



Chronic atrophic gastritis and pernicious anaemia

Persisting AIG eventually leads to atrophy of the affected areas. Besides AIG, chronic atrophic gastritis can also be caused by a *Helicobacter pylori* infection or by alcohol or pharmaceutical drug abuse. However, in contrast to these causes, the damage resulting from AIG is limited to the fundus and corpus of the stomach as a result of AIG.

The destruction of the parietal cells leads to hypo- or achlorhydria. Gastric acid is an important factor especially in the absorption of iron. Young patients in particular often develop iron deficiency anaemia, which cannot be treated by the additional oral administration of iron. Due to the loss of parietal cells, there is also a lack of intrinsic factor. The long-term consequence is vitamin B_{12} deficiency, leading to PA. The typical symptoms of anaemia such as fatigue, drowsiness, pallor and tachycardia are accompanied by diarrhoea, anorexia, glossitis, icterus and neurological symptoms.[4]

Autoimmune gastritis
- APCA
- Beginning destruction of parietal cells
- asymptomatic

Chronic atrophic gastritis
- Less APCA
- Possibly anti-intrinsic factor antibodies
- Atrophy of the stomach mucosa
- Malabsorption of iron and vitamin B_{12}

Pernicious anaemia
- Vitamin-B_{12} deficiency
- Anti-intrinsic factor antibodies
- Malabsorption, diarrhoea
- Fatigue, drowsiness, neurological symptoms

Antibodies

APCA are considered as the most sensitive biomarker for AIG. They can be detected in 80 to 90% of patients.[3] They mainly occur in the early stages of the disease and often several years before the onset of the first symptoms. In advanced gastritis, APCA are less frequently found, since the reduction in the number of parietal cells is accompanied by a decrease in the antigen. Due to the fact that APCA are also associated with other autoimmune diseases, e.g. Hashimoto's thyroiditis, diabetes mellitus type I or coeliac disease, they have a limited specificity. Moreover, they can be detected in around 20% of gastritis patients with *H. pylori* infection. APCA are rarely found in the healthy population, although their prevalence increases with age.

Antibodies against intrinsic factor are highly specific for AIG/PA, although their prevalence is lower than that of APCA. Whereas anti-intrinsic factor antibodies can be detected in the gastric juice of 80% PA patients, they are only found in the serum of 40 to 60% of patients, in long-term disease up to 80%.[3,4] Two types (both IgG) of anti-intrinsic factor antibodies in the serum can be distinguished. Type 1 blocks the vitamin B_{12} binding site of the intrinsic factor and type 2 inhibits the binding of the intrinsic factor/vitamin B_{12} complex to its corresponding receptors in the ileum. Type 1 IF antibodies are the most common and occur in 70% of PA patients. Type 2 IF antibodies are found in 30 to 40% patients, predominantly in combination with type 1 IF antibodies.[3,4]

Diagnostics with EUROIMMUN products

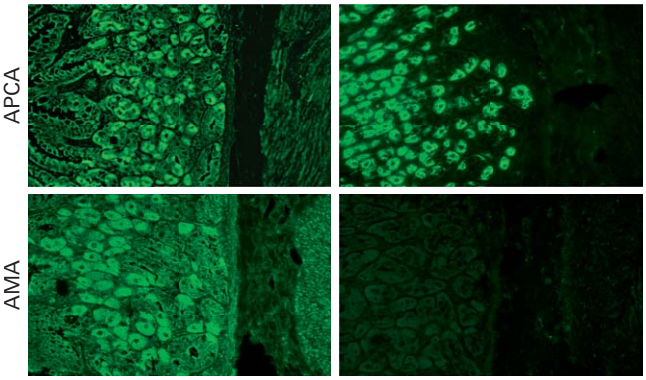
The diagnosis of AIG or PA is based on the detection of megaloblastic anaemia, a low vitamin B_{12} level in the serum, atrophy of the mucous membranes in the stomach fundus and corpus as well as antibodies against APCA and intrinsic factor.

IIFT: Stomach (Monkey) & EUROPLUS Intrinsic Factor

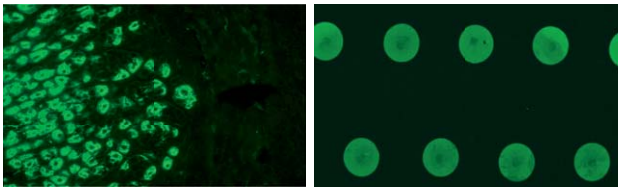
The indirect immunofluorescence test (IIFT) is used for sensitive detection of APCA. Primate stomach as the substrate ensures a higher specificity than the commonly used rat stomach. EUROIMMUN therefore recommends using primate stomach for investigation of APCA.

By pretreatment of the substrate with urea, the binding of possible anti-mitochondrial antibodies (AMA) is prevented, which facilitates the interpretation of results. In a positive reaction, only the cytoplasm of the parietal cells fluoresces. The fluorescing structures are fine granular to coarse clumpy.

Parallel incubation of primate stomach and EUROPLUS intrinsic factor dots with patient serum enables detection of APCA and the monospecific determination of anti-intrinsic-factor antibodies at the same time.



Without urea pretreatment | With urea pretreatment



APCA | Autoantibodies against intrinsic factor

Anti-PCA ELISA

The EUROIMMUN Anti-PCA ELISA allows simple quantitative determination of APCA titers. 190 uncharacterised sera from suspected AIG cases were investigated with the Anti-PCA ELISA and the IIFT: Stomach (Monkey). The specificity of the ELISA amounted to 94.0% with a sensitivity of 97.3% with respect to the IIFT.

Serum panel (n = 190)		IIFT: Stomach (Monkey)	
		positive	negative
Anti-PCA ELISA	positive	71	7
	negative	2	110

Anti-ATP4B ELISA

By using the highly recombinant β-subunit of H+/K+-ATPase, the EUROIMMUN Anti-ATP4B ELISA yielded an increase in the specificity compared to the Anti-PCA ELISA. In a panel of 100 healthy blood donors, the test showed a specificity of 99%, whereas the specificity of the Anti-PCA ELISA amounted to 93%.

The specificity of the Anti-ATP4B ELISA for 160 samples from patients with different other autoimmune diseases was 95.0%. In a study of 29 samples from clinically precharacterised AIG patients, the Anti-ATP4B ELISA yielded a sensitivity of 96.6%.

Healthy blood donors (n = 100)	Specificity
Anti-PCA ELISA (IgG)	93%
Anti-ATP4B ELISA (IgG)	99%

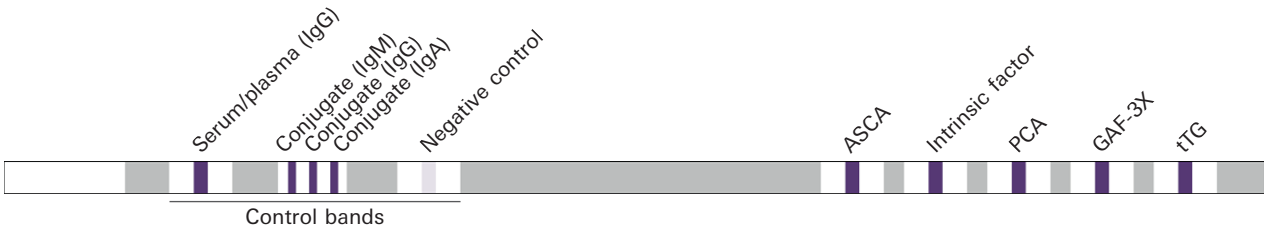
Panel	n	Anti-ATP4B positive (IgG)
Crohn's disease	30	1 (3.3%)
Ulcerative colitis	30	1 (3.3%)
Diabetes mellitus type I	30	3 (10.0%)
Autoimmune thyroiditis	20	1 (5.0%)
Coeliac disease	30	0 (0.0%)
Sjögren's syndrome	20	2 (10.0%)
Total (specificity)	**160**	**8 (95.0%)**

Anti-Intrinsic Factor ELISA

For confirmation of a positive result for anti-intrinsic factor antibodies in IIFT the additional analysis by means of ELISA is useful. The ELISA test system achieves a specificity and sensitivity of 100% compared to the radioimmunoassay.

EUROLINE Autoimmune Gastrointestinal Diseases (IgG)

The EUROLINE Autoimmune Gastrointestinal Diseases (IgG) enables the detection of antibodies against tissue transglutaminase (tTG), gliadin-analogous fusion peptide (GAF-3X), parietal cell antigen (PCA), intrinsic factor and mannan from *Saccharomyces cerevisiae* (ASCA) on a single blot strip. This supports the differentiation between coeliac disease, AIG or PA, and the chronic intestinal disease Crohn's disease in the case of otherwise unspecific symptoms.



In a nutshell

- The detection of APCA and autoantibodies against intrinsic factor significantly supports the diagnostics of AIG and PA.
- APCA can already be detected in early asymptomatic stages of AIG and have a high diagnostic sensitivity.
- Antibodies against intrinsic factor are highly specific and occur predominantly in the late disease stages.
- EUROIMMUN offers test systems for each diagnostic strategy. IIFT with primate stomach for initial detection of APCA, EUROPLUS intrinsic factor dots for parallel detection of anti-intrinsic factor antibodies, and the Anti-PCA, Anti-ATP4B and Anti-Intrinsic Factor ELISAs for confirmation and quantification of the test results. The EUROLINE Autoimmune Gastrointestinal Diseases (IgG) facilitates the differentiation between diseases with unspecific symptoms.

Order information

Test method	Test system	Antibodies against	Substrate	Order number
IIFT	IIFT: Stomach (Monkey)	Parietal cell antigen (PCA)	Tissue sections of primate stomach	FA 1360-####
	Anti-Intrinsic Factor IIFT	Intrinsic factor	Highly purified intrinsic factor	FA 1362-1005
	EUROPLUS Stomach (Monkey) / Intrinsic Factor	PCA, intrinsic factor	Tissue sections of primate stomach and highly purified intrinsic factor	FA 1362-####-1
ELISA	Anti-PCA ELISA (IgG)	H^+/K^+-ATPase	Native highly purified H^+/K^+-ATPase from porcine stomach mucosa	EA 1361-9601 G
	Anti-ATP4B ELISA (IgG)	β-Subunit of H^+/K^+-ATPase	Recombinant highly purified β-subunit of H^+/K^+-ATPase	EA 1361-9601-1 G
	Anti-Intrinsic Factor ELISA (IgG)	Intrinsic factor	Native highly purified intrinsic factor from porcine stomach mucosa	EA 1362-9601 G
EUROLINE	EUROLINE Autoimmune Gastrointestinal Diseases (IgG)	tTG, gliadin (GAF-3X), PCA, intrinsic factor and mannan from *S. cerevisiae*	Recombinant tTG, recombinant GAF-3X, native PCA from porcine stomach mucosa, recombinant intrinsic factor, native mannan from *S. cerevisiae*	DL 1360-#### G

References

[1]Toh B. **Diagnosis and classification of autoimmune gastritis.** Autoimm Rev 13: 459-462 (2014).
[2]Kulnigg-Dabsch S. **Autoimmune gastritis.** Wien Med Wochenschr 166: 424-430 (2016).
[3]Rusak E et al. **Anti-parietal cell antibodies – diagnostic significance.** Adv Med Sci 61: 175-179 (2016).
[4]Bizarro N, Antico A. **Diagnosis and classification of pernicious anemia.** Autoimm Rev 13: 565-568 (2014).