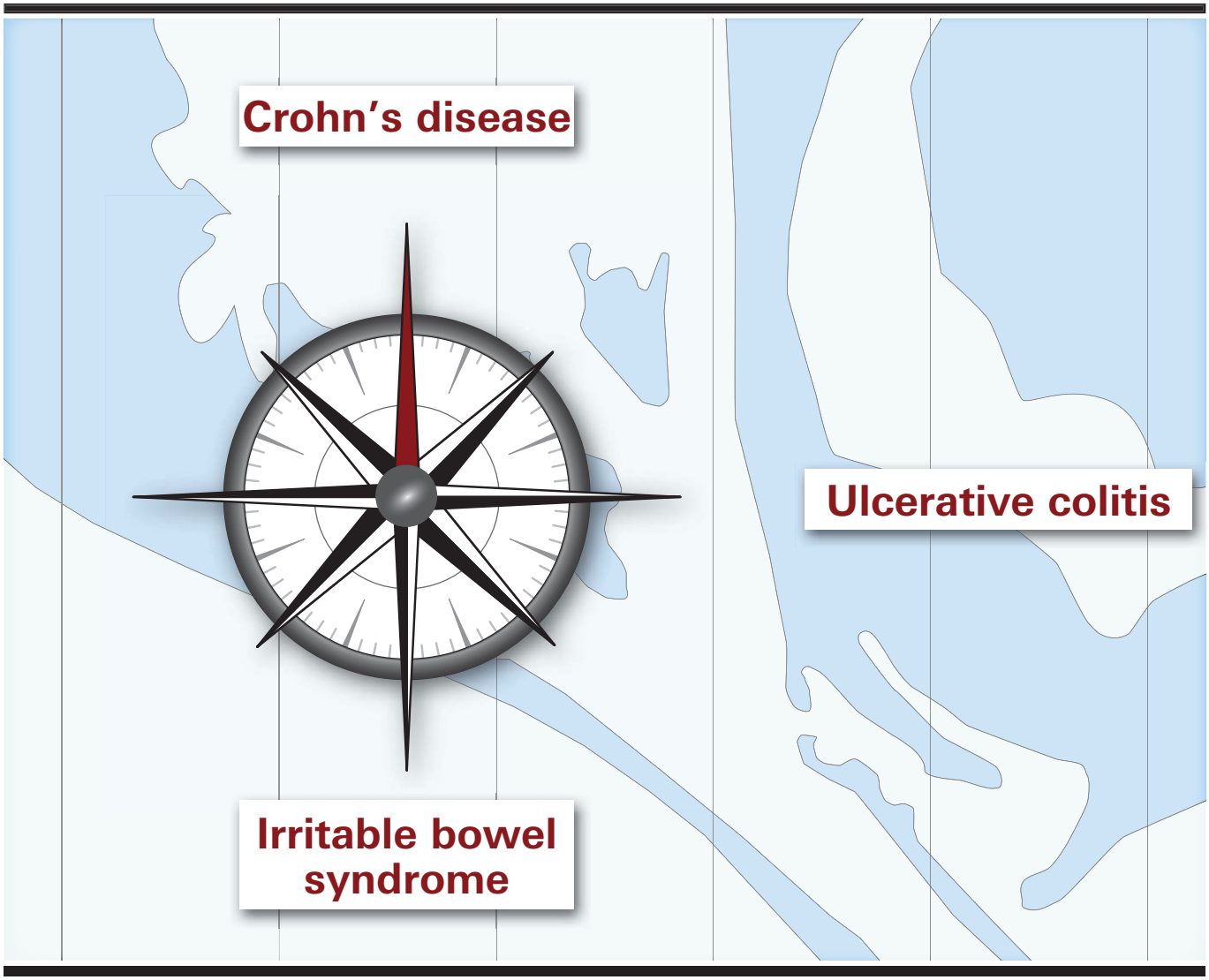
EUROIMMUN Medizinische Labordiagnostika AG

# Chronic inflammatory bowel diseases

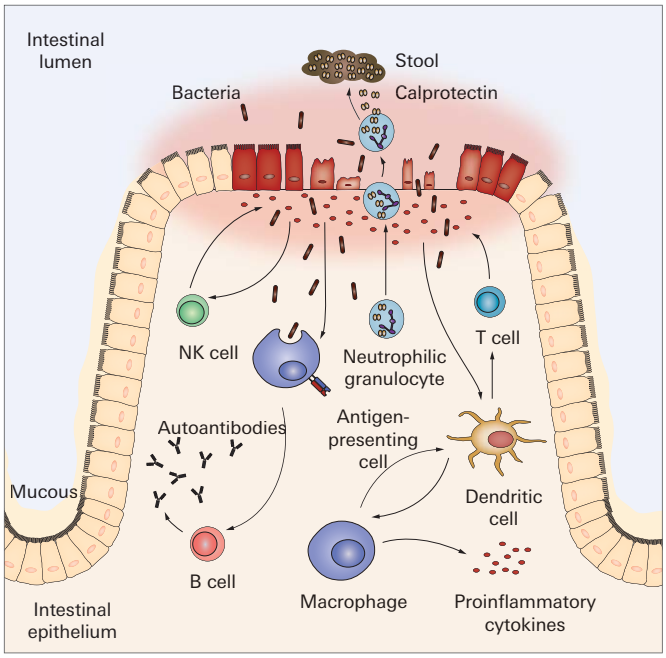
## Faecal and serological markers to support the diagnosis of Crohn's disease and ulcerative colitis



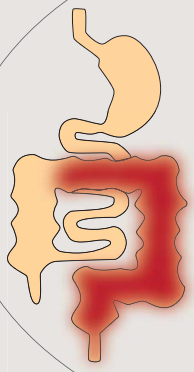

- **Calprotectin ELISA** – Secure differentiation between chronic inflammatory and functional bowel diseases
- **IIFT CIBD Mosaics** – Reliable screening tests for efficient differential diagnosis of ulcerative colitis and Crohn's disease
- **Anti-Saccharomyces cerevisiae ELISA** – Provides useful information on the severity of the diseases
- **EUROLINE Autoimmune Gastrointestinal Diseases** – Discrimination of Crohn's disease from coeliac disease and autoimmune gastritis

# Chronic inflammatory bowel diseases

Chronic inflammatory bowel diseases (CIBD) are characterised by inflammation in different areas of the gastrointestinal tract and occur in episodes. Symptomatic phases (relapses) alternate with remission phases, in which the disease is inapparent. The severity of the symptoms and the duration of the episodes differ from patient to patient. The aetiology of CIBD is still relatively unknown. But it is assumed that genetic susceptibility and certain environmental factors (antibiotic treatment, smoking, "western diet") can cause the disease. The most relevant CIBD forms are ulcerative colitis (UC) and Crohn's disease (CD). In around 10% of cases, a mixed form is observed, which cannot be clearly assigned to either of the diseases (indeterminate colitis).[1] Both in CD and UC there is a dysfunction of the intestinal barrier of mucosa and intestinal epithelium. Thus, pathogenic bacteria may travel from the intestinal lumen to the epithelial cells and trigger an inflammatory response. For differentiation between the different CIBD and for discrimination of these from irritable bowel syndrome, targeted differential diagnostics are of great importance.[2,3]




## Ulcerative colitis

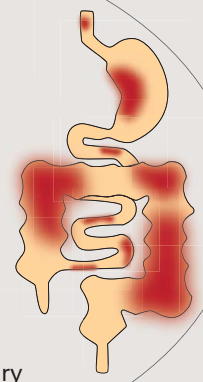


- Incidence: 3.0–24 per 100,000[4]
- Prevalence: 90–500 per 100,000[4]
- Manifestation before age 18: 20–30% of patients[4]
- Latency period until diagnosis: 1.2 years[6]
- Colectomy necessary in 10–30% of patients[7]

## Indeterminate colitis

## Crohn's disease

- Incidence: up to 12.7 per 100,000[5]
- Prevalence: up to 322 per 100,000[5]
- Manifestation before age 18: approx. 25% of patients[5]
- Latency period until diagnosis: 7.7 years[6]
- Intestinal surgery necessary in 80% of patients[7]



### Symptoms of UC:

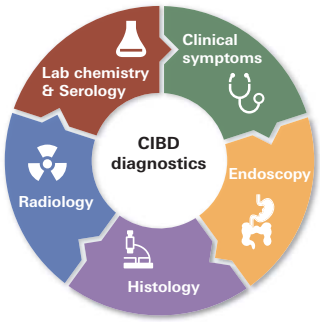
- The inflammation is often limited to the colon.
- It starts at the anus and spreads further along the colon.
- Only the intestinal mucosa is inflamed.
- Typical symptoms are bloody diarrhoea (mostly at night and postprandial) with pus or slimy discharge and severe abdominal cramps during defaecation.
- The most severe complications include toxic megacolon, which is defined by extreme dilatation of the colon and potential bursting.

### Symptoms of CD:

- The inflammation can affect the entire gastrointestinal tract – from the mouth to the anus.
- Patches of inflamed intestinal sections alternate with healthy patches, with the terminal ileum being most frequently affected.
- The symptoms often depend on the disease locus and resemble those of UC.
- The inflammation may permeate all the layers of the intestinal wall and spread even further. This can lead to the formation of fistulas and abscesses.
- Scarred and swollen intestinal tissue can encourage intestinal stenosis.

# Faecal and serological markers of chronic inflammatory bowel diseases

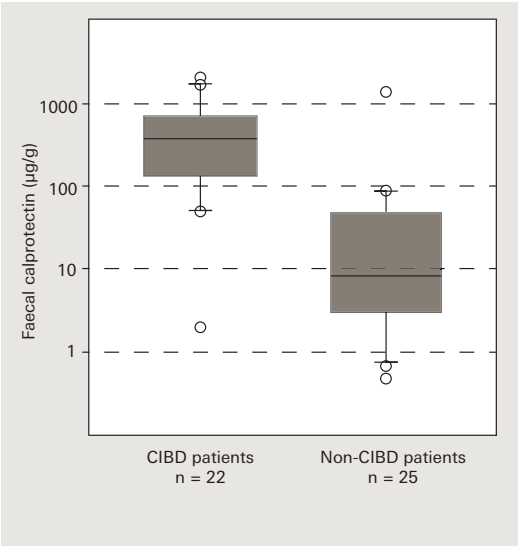
The diagnosis of CIBD is based on the clinical picture of the patient as well as on a combination of laboratory, endoscopic, histological and radiological tests. The **faecal inflammation marker calprotectin** is an important parameter in laboratory testing. In addition to early diagnosis, it enables discrimination of CIBD from functional bowel diseases such as irritable bowel syndrome. The additional **detection of CIBD-associated autoantibodies (IgA and IgG)** in serum can further secure the diagnosis. Indirect immunofluorescence tests (IIFT), enzyme-linked immunosorbent assays (ELISA) and blot-based tests can be used to determine markers that are pathognomonic for CD or UC, thus allowing differentiation between the two diseases.




## Calprotectin – a faecal CIBD marker

Calprotectin is a calcium- and zinc-binding protein complex, which is produced by neutrophilic granulocytes and monocytes. In case of an inflammatory intestinal disease, neutrophils move into the gut lumen and release calprotectin, which is secreted with stool. In CIBD diagnostics, faecal calprotectin (FC) is the ideal marker for various reasons.

- Calprotectin is produced at the onset of the intestinal inflammation and therefore allows early diagnosis.
- Increased FC values exclusively reflect inflammatory processes in the gastrointestinal tract. FC is therefore a much better marker for the diagnosis of CIBD than systemic markers such as C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR).
- A positive FC result is used to discriminate CIBD from a functional disease such as irritable bowel syndrome. Concentrations >50 µg/g should be considered conspicuous. The inflammation status should be verified by endoscopy. However, the determination of FC cannot be used to differentiate between CD and UC.
- The FC concentration is proportional to the number of neutrophilic granulocytes in the intestinal lumen – and thus to the severity of the inflammation – and correlates with the activity of the CIBD (relapse or remission). It therefore supports assessment of the severity of the disease and helps to reduce the number of endoscopies and biopsies, which are costly and often stressful for the patient. This is a particular advantage in paediatrics.



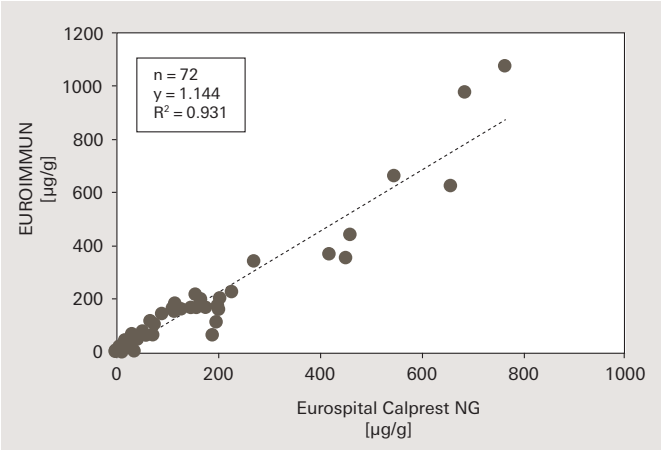

All current guidelines on CIBD diagnostics recommend the detection of FC for establishing a diagnosis. Especially the good correlation of the marker with the inflammation activity is emphasised. Moreover, FC levels can support the prediction of relapses.

| Calprotectin in international guidelines on CIBD diagnostics | | | | | | | |
|---|---|---|---|---|---|---|---|
| Year | 2015 | 2018 | | | 2019 | 2020 | 2021 |
| Organisation | WGO[8] | JSGE[9] | ACG[10] | ECCO[11] | BSG[12] | DGVS[2] | DGVS[3] |
| Country/Region | Worldwide | JP | USA | EU | UK | D | D |
| Disease | CIBD | CIBD | CD | CIBD | CIBD | UC | CD |
| Differential diagnostics for CIBD / IBS | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Correlation with disease activity | □ | □ | □ | ■ | ■ | ■ | ■ |
| Prognosis of a relapse | | | □ | ■ | ■ | ■ | ■ |
| Marker of mucosal healing | | | | ■* | | | □ |
| Marker of post-operative relapse | | | | ■ | ■ | | ■ |

■ recommended; □ mentioned; * only UC

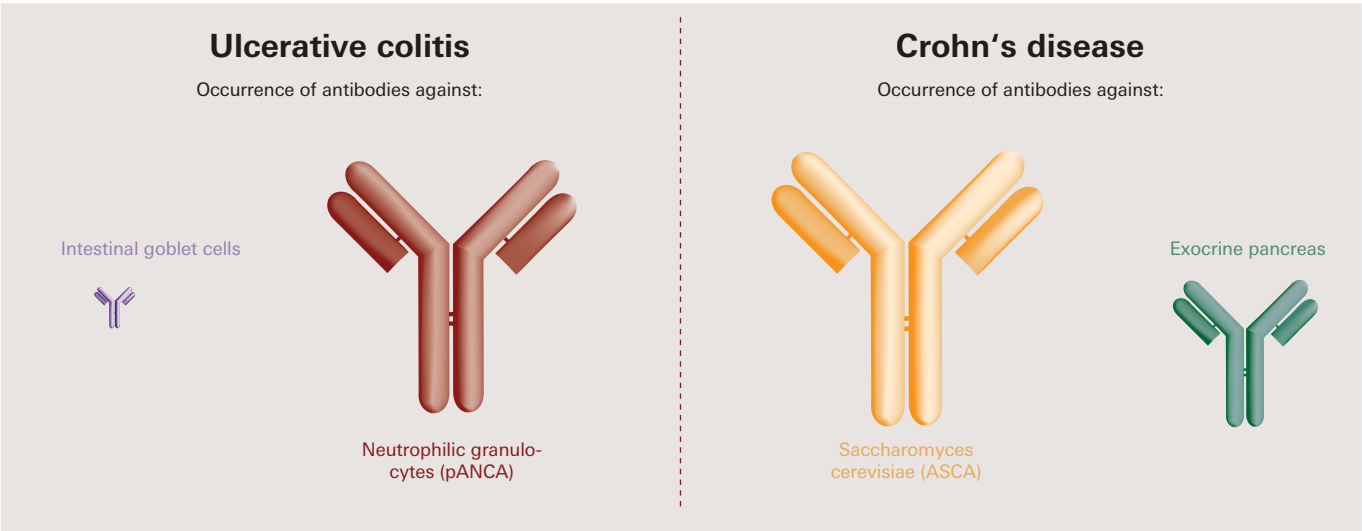
EUROIMMUN offers the Calprotectin ELISA for reliable measurement of FC in stool samples:

- Non-invasive, quantitative determination of the FC level
- Rapid and simple processing in approx. 75 min
- Comprehensive measurement range of 1.9–2100 µg/g
- Good correlation with other established assays such as the Calprotectin ELISA from Eurospital
- Fully automatable on all open ELISA platforms
- Stool dosage tubes for convenient sample extraction available

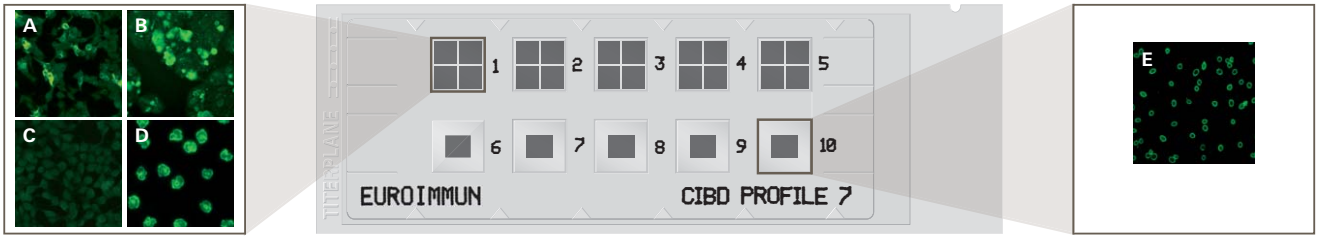



## Serological markers for the diagnosis of CIBD

Chronic inflammatory bowel diseases can be diagnosed in most patients by serologically testing for various pathognomonic antibodies. Determination of these antibodies supports the differential diagnosis and allows conclusions to be drawn with respect to the presence of UC or CD (in the following figure, the proportions of the antibodies represent the different frequencies of occurrence).



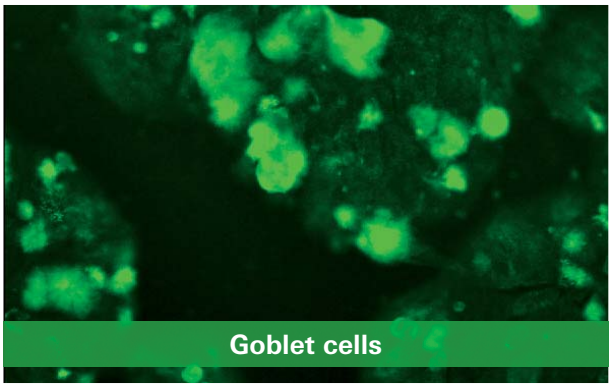

**Indirect immunofluorescence test (IIFT):** EUROIMMUN produces BIOCHIP combinations with various IIFT substrates, which are used to detect specific autoantibodies against intestinal goblet cells, exocrine pancreas, anti-neutrophil cytoplasmic antibodies (ANCA) and antibodies against *Saccharomyces cerevisiae* (ASCA). The slides for the IIFT Mosaics (e.g. CIBD profiles) can be flexibly equipped with different BIOCHIPs. As an example, the CIBD Profile 7 is depicted:




A: Autoantibodies against exocrine pancreas antigens: rPAg1 (CUZD1)/ rPAg2 (GP2); B): Antibodies against goblet cells; C: Control-transfected cells; D: Anti-neutrophil cytoplasmic antibodies, perinuclear type (pANCA); E: Antibodies against *Saccharomyces cerevisiae*.

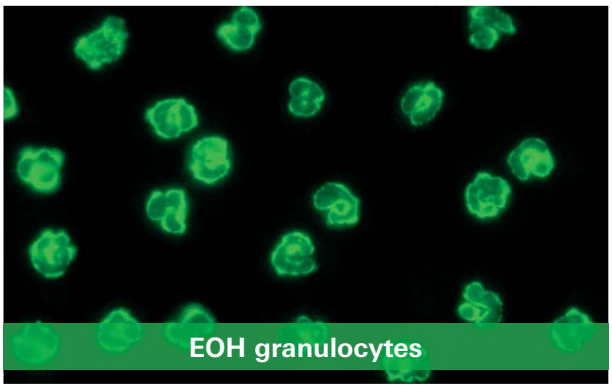
## Antibodies against intestinal goblet cells

**IIFT:** Autoantibodies directed against intestinal goblet cells are pathognomonic for UC and occur in 11 % of patients.[13,14] The relevant target antigen has not yet been identified. The BIOCHIP uses a primate intestinal cell line, whose cells differentiate spontaneously into goblet cells under defined culture conditions. In case of a positive reaction in the IIFT, the substrate shows a cloudy fluorescence with fuzzy borders. When both antibodies against goblet cells and pANCA are investigated, the combined detection rate for UC amounts to 82 %.



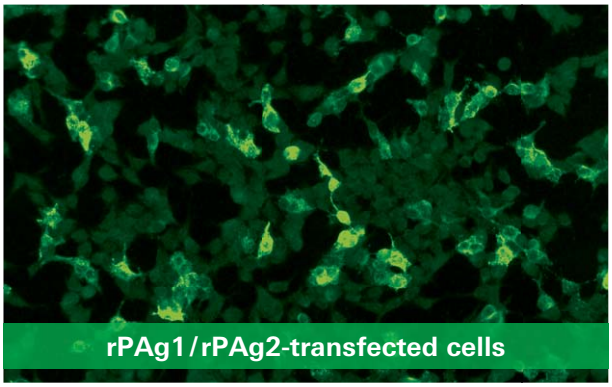

## Anti-neutrophil cytoplasm antibodies (ANCA)

**IIFT:** ANCA of the perinuclear type (pANCA) are not only of major importance in the serological diagnosis of various forms of vasculitis, but also in the differential diagnosis of CIBD. They occur in the majority of patients with UC (67 %), but can also be found in some CD patients (7 %).[15] Patients with CIBD produce a pANCA pattern on ethanol-fixed granulocytes, whereas formalin-fixed cells do not react. This atypical form of ANCA is called DNA-ANCA (or aANCA or xANCA) in literature.




## Antibodies against exocrine pancreas

**IIFT:** Autoantibodies against exocrine pancreas are directed against acinar cells. They can be found in 39 % of patients with CD, while they are only present in around 2 % of UC patients. They are therefore characteristic of CD.[16,17] In CD, the target antigens of the autoantibodies are the protoglycans CUZD1 (PAg1) and GP2 (rPAg2) in the pancreas secretion.[17] These antigens are detected using transfected cells, which ensure reproducibility of the results. The prevalence of IgG autoantibodies against CUZD1 and GP2 is on average 40 %, in CD of a duration of more than two years even 50 %. Antibodies against rPAg1 cause a broad granular fluorescence in the cytoplasm, the cell nuclei are only weakly stained. Anti-rPAg2 antibodies stain the cytoplasm with a smooth to fine-granular fluorescence that is mainly located in the nucleus. By investigating autoantibodies against pancreas antigens as well as ASCA in CD diagnostics, the detection rate for CD can be increased to 80 %.[18,19]

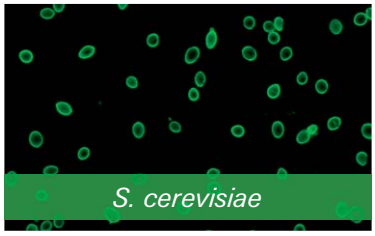


## STUDY

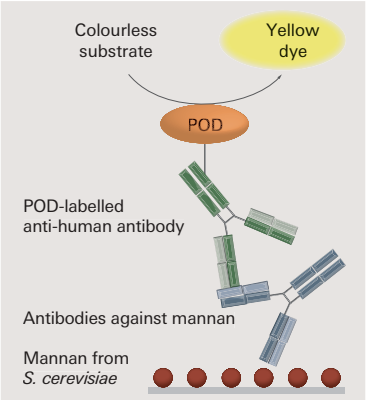
Autoantibodies against the pancreas antigens CUZD1 and GP2 have a high specificity for the diagnosis of CD. Michaels et al. showed in a study based on 224 CD and 136 UC patients that each of these autoantibodies is associated with a specific clinical phenotype.[20] Autoantibodies against GP2 and CUZD1 were both found significantly more often in CD than in UC patients. Generally, antibodies against these two antigens were associated with the presence of ASCA and the necessity of immunosuppressive therapy. Patients with autoantibodies against CUZD1 suffered significantly more often from ileocolic and perianal diseases. In patients with anti-GP2 antibodies, intestinal strictures were especially frequently observed. Patients with anti-CUZD1 or anti-GP2 antibodies were younger at the onset of the disease than those who did not exhibit these antibodies.

| n = 360 | Antibodies against pancreatic glycoproteins | |
|---|---|---|
| | Antibody-positive | Antibody-negative |
| Ileocolic disease | 44.1 % | 23.4 % |
| Perianal disease | 48.6 % | 31.1 % |
| ASCA | 43.1 % | 29.6 % |
| Immunosuppressives | 43.6 % | 30.0 % |
| | Anti-CUZD1 antibodies | |
| Age at onset | 19.5 years | 27.5 years |
| Ileocolic disease | 30.3 % | 15.6 % |
| Perianal disease | 37.8 % | 18.9 % |
| | Anti-GP2 antibodies | |
| Age at onset | 20 years | 26 years |
| Intestinal strictures | 25.3 % | 12.2 % |

## Anti-*Saccharomyces cerevisiae* antibodies (ASCA)




**IIFT:** ASCA mostly occur in CD patients. ASCA of classes IgA and IgG together have a prevalence of around 73 %.[19] In UC patients, ASCA occur less frequently with a prevalence of around 18 %.[13] The detection of ASCA therefore supports the diagnostic differentiation of CD from UC. A yeast smear is used as the IIFT substrate. The main antigen of ASCA is phosphopeptidomannan, which is a 200 kDA glycoprotein from the yeast cell wall. Antibodies against *Saccharomyces cerevisiae* cause a broad to edge-accentuated fluorescence of the yeast cells.




**ELISA:** ASCA can also be reliably detected by means of ELISA. With the EUROIMMUN Anti-Saccharomyces cerevisiae ELISA (IgA, IgG) they can be investigated in serum or plasma. The break-off microplate wells of the test system are coated with mannan, a carbohydrate from the yeast cell wall. In positive samples, specific IgA or IgG antibodies bind to the antigens. To make ASCA visible, the samples are then incubated with a peroxidase (POD)-labelled anti-human antibody. POD catalyses a colour reaction, which can be measured using a photometer.

The prevalence of ASCA in a clinically precharacterised cohort of 67 CD patients was 43.3 % for IgA and 31.3 % for IgG antibodies. The Anti-Saccharomyces cerevisiae ELISA yielded a specificity of 100 % in a precharacterised UC control cohort (n = 47).

## STUDIES

In a study with 115 CD patients, Kim et al. investigated whether the presence of ASCA is associated with a specific disease course.[21] CD patients exhibiting ASCA showed the following characteristics:

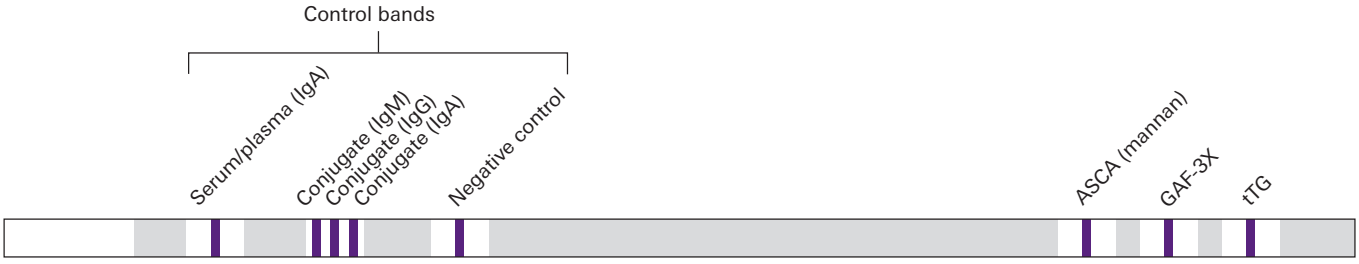
- increased fibrostenosis and intestinal ruptures (according to the Vienna classification),
- more frequent hospitalisation,
- increased values according to the Harvey-Bradshaw index (a scale for quantification of the disease activity in CIBD).

Moreover, steroids and immunosuppressives for treatment were used more frequently. In CD, ASCA may therefore indicate a severe disease course, which requires more aggressive therapy.

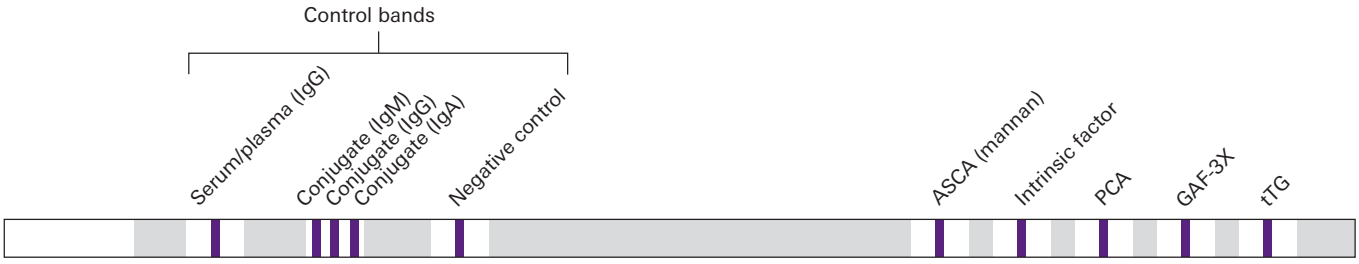
A prospective study by Kovacs et al. investigated whether there is an association between ASCA and the activity of UC.[13] A total of 187 clinically diagnosed UC patients (origin: Hungary), 17.6 % of them positive for ASCA, were monitored over a period of 135 months. ASCA IgA-positive patients had an increased risk of requiring long-term immunosuppressive therapy.

## EUROLINE test systems for autoimmune gastrointestinal diseases

**Blot:** Differentiation between CD and other autoimmune diseases of the gastrointestinal tract such as coeliac disease and autoimmune gastritis (AIG) or pernicious anaemia (PA) is difficult due to the unspecific clinical symptoms. Serological determination of disease-specific antibodies is particularly useful in these cases for efficient diagnostics. Antibodies against tissue transglutaminase (tTG) occur in coeliac disease with a prevalence of almost 100 %, whereas they are virtually absent in healthy persons and patients with other intestinal diseases. Furthermore, patients with coeliac disease form very specific antibodies against deamidated gliadin fragments. In the blood of AIG or PA patients, antibodies against intrinsic factor (IF) and parietal cell antigens (PCA) are present. The presence of ASCA, however, is indicative of CD. Thus, parallel detection of these antibodies provides important information for differential diagnosis. For this purpose, EUROIMMUN offers two EUROLINE test systems. The test strips are coated with recombinant tTG, recombinant gliadin-analogue fusion peptide (GAF-3X) and mannan from *S. cerevisiae*. Additionally, the EUROLINE Autoimmune Gastrointestinal Diseases (IgG) contains recombinant IF as well as native PCA:




EUROLINE Autoimmune Gastrointestinal Diseases (IgA)




EUROLINE Autoimmune Gastrointestinal Diseases (IgG)

## In a nutshell

- For CIBD diagnostics, endoscopic, histological and radiological examinations should be supplemented by laboratory and serological tests.
- The determination of calprotectin in stool allows early diagnosis and differentiation between CIBD and irritable bowel syndrome. Normal calprotectin values largely exclude CIBD.
- According to current guidelines, calprotectin levels are a valuable aid in monitoring the disease activity and therapy control. Calprotectin values correlate with the clinical result from endoscopy or biopsy. The determination is a non-invasive and, particularly for paediatric patients, favourable examination method.
- The investigation of patient sera by IIFT supports the reliable differentiation of a MC from a CU. It is non-invasive and less costly than other methods.
- Specific markers for the diagnosis of UC are antibodies against intestinal goblet cells und pANCA. Antibodies against exocrine pancreas antigens and ASCA point towards CD.
- The combination of different IIFT substrates in one mosaic increases the detection rate in differential CIBD diagnostics.
- In a study by Michaels *et al.*, antibodies against CUZD1 were associated with ileocolic and perianal diseases, and antibodies against GP2 with intestinal strictures.[20] Both antibodies were accompanied by ASCA and the necessity of immunosuppressive therapy.
- The presence of ASCA indicates a severe course of CD. In UC patients, they are associated with an increased risk of requiring long-term therapy with immunosuppressives.
- For exclusion of other autoimmune gastrointestinal diseases, such as coeliac disease and autoimmune gastritis, antibodies that are highly relevant in differential diagnostics are investigated: antibodies against tTG, deamidated gliadin fragments, IF and PCA.

## Ordering

| Test system | Antigen | Substrate | Bestell-Nr. |
|---|---|---|---|
| Calprotectin ELISA | calprotectin | antibody-coated microplate wells | EQ 6831-9601 W |
| Stool dosage tubes (SDT), prefilled with extraction buffer, 45 pieces | – | – | ZE 6010-4501 |
| Stool dosage tubes (SDT), not filled with extraction buffer, 100 pieces | – | – | ZE 6010-0100-1 |
| **Test system** | **Antibodies against** | **Substrate/Antigen** | **Bestell-Nr.** |
| CIBD mosaics | intestinal goblet cells | goblet cells (culture) | FA 1391-1005-3<br>FA 1391-1005-7 |
| | pancreas antigens rPAG1 (CUZD1)/rPAg2 (GP2) | transfected cells | |
| | pANCA | granulocytes (EOH) | |
| | *S. cerevisiae* | fungal smear | |
| Anti-Saccharomyces cerevisiae IIFT (IgA, IgG) | *S. cerevisiae* | fungal smear | FV 2841-1010 A/G |
| Anti-Saccharomyces cerevisiae ELISA (IgA, IgG) | *S. cerevisiae* | purified mannan from *S. cerevisiae* cell wall | EV 2841-9601 A/G |
| **Test system** | **Antibodies against** | | **Bestell-Nr.** |
| EUROLINE Autoimmune Gastrointestinal Diseases (IgA) | tTG, GAF-3X, mannan from *S. cerevisiae* | | DL 1360-1601 A |
| EUROLINE Autoimmune Gastrointestinal Diseases (IgG) | tTG, GAF-3X, PCA, IF, mannan from *S. cerevisiae* | | DL 1360-1601 G |

## References

[1] Deutsche Morbus Crohn/Colitis ulcerosa Vereinigung (DCCV e.V.); www.dccv.de. [2] Kucharzik T, et al. **Aktualisierte S3-Leitlinie Colitis ulcerosa.** Z Gastroenterol. 58:241-326 (2020) [In German]. [3] Sturm A, et al. **Aktualisierte S3-Leitlinie – „Diagnostik und Therapie des Morbus Crohn" der Deutschen Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten (DGVS).** Z Gastroenterol. 60:332-418 (2022) [In German]. [4] Conrad K, et al. **Diagnosis and classification of ulcerative colitis.** Autoimmun Rev. 13:436-466 (2014). [5] Laass M, et al. **Diagnosis and classification of Crohn's disease.** Autoimmun Rev. 13:467-471 (2014). [6] Pimentel M, et al. **Identification of a prodromal period in Crohn's disease but not ulcerative colitis.** Am J Gastroenterol 95:3458-3462 (2000). [7] Cosnes J, et al. **Epidemiology and natural history of inflammatory bowel disease.** Gastroenterology 140:1785-1794 (2011). [8] Bernstein C, et al. **World gastroenterology organisation global guidelines inflammatory bowel disease: Update August 2015.** J Clin Gastroenterol 50:803-818 (2016). [9] Matsuoka K, et al. **Evidence-based clinical practice guidelines for inflammatory bowel disease.** J Gastroenterol 53:305-353 (2018). [10] Lichtenstein GR, et al. **ACG Clinical Guideline: Management of Crohn's Disease in Adults.** Am J Gastroenterol 113:481-517 (2018). [11] Maaser C, et al. **ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications.** J Crohns Colitis 13(2):144-164 (2019). [12] Lamb CA, et al. **British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults.** Gut 68: s1-s106 (2019). [13] Kovacs G, et al. **Significance of serological markers in the disease course of ulcerative colitis in a prospective clinical cohort of patients.** PLoS ONE 13:e0194166 (2018). [14] Homsak E, et al. **Autoantibodies pANCA, GAB and PAB in inflammatory bowel disease: prevalence, characteristics and diagnostic value.** Wien Klin Wochenschr 122:19-25 (2010). [15] Stöcker W, et al. **Autoantibodies to granulocytes in chronic inflammatory bowel disease are not correlated with antibodies to intestinal goblet cells in ulcerative colitis and to pancreatic juice in Crohn's disease.** Immunobiology 186:96 (1992). [16] Stöcker W, et al. **Autoimmunity to pancreatic juice in Crohn's disease. Results of an autoantibody screening in patients with chronic inflammatory bowel disease.** Scand J Gastroenterol 139:41-52 (1987). [17] Komorowski L, et al. **Autoantibodies against exocrine pancreas in Crohn's disease are directed against two antigens: The glycoproteins CUZD1 and GP2.** Journal of Crohn's and Colitis 7:780-790 (2013). [18] Teegen B, et al. **Prevalence of antibodies against *Saccharomyces cerevisiae* in the diagnosis of chronic-inflammatory bowel disease.** J Lab Med 24:494 (2000). [19] Kovacs M, et al. **Pancreatic autoantibodies and autoantibodies against goblet cells in pediatric patients with inflammatory bowel disease.** JPGN 55:429-435 (2012). [20] Michaels MA, et al. **Pancreatic autoantibodies against CUZD1 and GP2 are associated with distinct clinical phenotypes of Crohn's disease.** Inflamm Bowel Dis 21:2864-2872 (2015). [21] Kim BC, et al. **Clinical significance of anti-*Saccharomyces cerevisiae* antibody (ASCA) in Korean patients with Crohn's disease and its relationship to the disease clinical course.** Dig Liver Dis 39:610-616 (2007).