Indications: Test system for the in vitro determination of antibodies against intestinal goblet cells in human serum or plasma for the diagnosis of the following disease: ulcerative colitis.

Clinical significance: Autoantibodies against intestinal goblet cells exclusively occur in ulcerative colitis (UC) and they are most likely the expression of a pathogenetically relevant autoimmunity. Similarly, disease-specific autoantibodies are also found in the second chronic inflammatory bowel disease, Crohn's disease. These autoantibodies are directed against a secretion product of the pancreas (pancreas acinus cell antibodies) and probably play a role in the onset of the disease.

The disease localization is reflected macroscopically as well as microscopically in the distribution of the goblet cells: there are only a few goblet cells in the duodenum, whereas their number increases continually towards the rectum. Therefore, in ulcerative colitis the duodenum is never affected. The disease develops in the rectum and spreads upwards with increasing disease activity. In the crypts of the colon there is a high concentration of goblet cells. In biopsy examinations cryptitis is always a sign of ulcerative colitis, whereas it is rare in Crohn's disease. The target antigen responsible for ulcerative colitis has not yet been exactly identified. The prevalence of positive results in UC is only 28% (Crohn's disease patients 0%, healthy individuals 0%), with a distribution of immunoglobulin classes as follows: IgA 8%, IgG 23%, IgA and IgG 69%. The prevalence of goblet cell antibodies in ulcerative colitis is higher in male patients (m:f=3.3:1), although this is not the case for pANCA (m:f=0.9:1).

The determination of antibodies against goblet cells, pANCA, antibodies against exocrine pancreas and antibodies against Saccharomyces cerevisiae can significantly enrich differential diagnostics of chronic inflammatory bowel diseases (Crohn's disease, ulcerative colitis). Many patients would be spared unpleasant diagnostic torture if clinicians would make more use of such significant diagnostics. Apparently, due to their unrivalled hit rate in gastroenterology (in positive results), autoantibody tests have to compete too much with endoscopical examinations.

Antibodies against granulocytes (pANCA, q.v. anti-neutrophil cytoplasmic antibodies) can also occur in ulcerative colitis as well as (less frequently) in Crohn's disease. They are detected by indirect immunofluorescence using smears of human ethanol-fixed granulocytes. They show a smooth, partly fine-granular, perinuclear fluorescence of the cytoplasm (pANCA) and do not react with formalin-fixed granulocytes. The same pANCA are found in primary sclerosing cholangitis, which is often associated with ulcerative colitis. DNA-bound lactoferrin could be identified as the main target antigen. The prevalence of these pANCA in UC is 67% (Crohn's disease 7%, healthy persons 0-1%), distribution of immunoglobulin classes: IgA 3%, IgG 39%, IgA and IgG 58%).

Application of the IIFT Intestinal Goblet Cells (Culture): For the first time a cultivated intestinal cell line is used as the antigen substrate in the EUROIMMUN IIFT Intestinal Goblet Cells (Culture). Conventional test systems generally use primate intestinal tissue for the detection of intestinal goblet cells. Due to natural variations of the tissue quality their reproducibility is only limited.

By using a cell culture for substrate production EUROIMMUN is able to offer continually good antigen quality in all EUROIMMUN IIFT Intestinal Goblet Cells (Culture), thus ensuring a reproducible reactivity. Unspecific reactions, which can be found occasionally in tissue substrates, have also been successfully reduced with the new substrate.
**Test characteristics**

**Intestinal Goblet Cells (Culture)**

**Principle of the test:** The indirect immunofluorescence test is an in vitro assay for the quantitative or qualitative determination of specific antigens against intestinal goblet cells. Prepared goblet cells are incubated with diluted patient samples. In the case of positive reactions, specific antibodies of the class IgA, IgG and IgM will bind to the antigens. In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and made visible with the fluorescence microscope.

**Reproducibility:** The intra-assay reproducibility was determined using 2 characterised samples which were incubated simultaneously and ten times each. The inter-assay reproducibility was also determined using 2 characterised samples which were incubated twice in a total of 5 different test runs on at least 2 different days. In both cases the results showed no deviations in the titre level when evaluated quantitatively.

**Clinical data:** When tested with samples containing antibodies against intestinal goblet cells (origin: Germany) the IIFT Intestinal Goblet Cells (Culture) showed a specificity of 100%. The sensitivity was also 100% (reference test: IIFT primate intestine as antigen substrate).

**Reference range:** In healthy blood donors (origin: Germany) the following antibody prevalences (IgA and IgG: titre 1:10 or higher) were determined (see table).

+++

**Technical data:**

- **Antigen substrate:** Intestinal goblet cells (culture).
- **Sample material:** Serum or plasma.
- **Sample dilution:** IgA and IgG
  - Qualitative evaluations: 1:10;
  - Quantitative evaluations: 1:10, 1:100, etc.
  - There is no upper limit to the measurement range.
- **Test procedure:** 30min (sample) / 30min (conjugate). Room temperature. Automatable.
- **Microscopy:** Objective 40x
  - Excitation filter: 488nm, colour separator: 510nm, blocking filter: 520nm
  - Light source: EUROIMMUN LED or mercury vapour lamp, 100 W.
- **Reagents:** Ready for use, with the exception of the PBS-Tween buffer (for dilution and washing).
- **Stability:** All kit components are stable for at least 18 months from the date of manufacture.
- **Kit formats:** 10 or 20 slides, each containing 3, 5 or 10 test fields. Kits include all necessary reagents.
- **Order no.:** FI 1381-#### A or G

**Incubation with the TITERPLANE™ Technique**

<table>
<thead>
<tr>
<th>Antibodies against</th>
<th>Prevalence IgA</th>
<th>Prevalence IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal goblet cells (culture) positive</td>
<td>11 (n = 200 blood donors)</td>
<td>0 (n = 200 blood donors)</td>
</tr>
<tr>
<td>Intestinal goblet cells (culture) negative</td>
<td>0</td>
<td>46</td>
</tr>
</tbody>
</table>

(See table for antibody prevalences in healthy blood donors and intestinal tissue samples.)