Indication: Test system for the in vitro determination of antibodies against parietal cells (PCA) and intrinsic factor in human serum or plasma for the diagnosis of the following diseases: chronic atrophic gastritis, pernicious anaemia (PA), funicular myelosis, various autoimmune endocrinopathies.

Clinical significance: Parietal cells (border cells, Exocrinocyti parietales) are located in the stomach mucosa, scattered between peptic cells, which produce pepsinogen (precursor of the enzyme pepsin). The relatively large parietal cells have one or two cell nuclei and an eosinophilic cytoplasm. They are responsible for hydrochloric acid production in the stomach and secretion of intrinsic factor, a glycoprotein which is required for the uptake of cyanocobalmin (vitamin B12) in the terminal part of the ileum. Some parietal cells also produce ghrelin, which regulates food ingestion and the secretion of growth hormones. PCA are directed against the enzyme H+K+-ATPase (proton pump), which is mainly involved in hydrochloric acid production.

In chronic atrophic gastritis the stomach mucosa is infiltrated by lymphocytes, plasma cells and granulocytes. The epithelial cells become necrotic, causing the peptic and parietal cells to be replaced by mucoid cells. In advanced stages of chronic atrophic gastritis, i.e. after several years, atrophy of the stomach mucosa develops, characterised by a continuous decrease in hydrochloric acid production. For all patients exhibiting antibodies against parietal cells who also underwent endoscopic examination, the serological diagnosis of “chronic atrophic gastritis” could be confirmed by the endoscopic result. The prevalence is almost 100% unless the stomach mucosa is not yet fully atrophic. As chronic atrophic gastritis is the highest risk factor for the development of gastric carcinoma, early diagnosis by PCA detection is of particular importance. In numerous investigations, a Helicobacter pylori infection was detected in 50% to 70% of chronic atrophic gastritis cases during the early stage of the disease. Hence the aetiological cascade Helicobacter pylori infection to chronic atrophic gastritis to gastric carcinoma is of great significance for the diagnostic and therapeutic approach as regards the serological detection of PCA and antibodies against Helicobacter pylori and suitable treatment. Patients with PA show symptoms of hyperchromic anaemia. Characteristic is the occurrence of megaloblasts in the bone marrow and megalocytes in the blood. The disease follows chronic atrophic gastritis and has therefore a causative link to primary factor deficiency and secondary vitamin B12 deficiency. The decrease in the prevalence of autoantibodies against parietal cells during the course of pernicious anaemia is of particular importance. This is likely to be caused by the permanent loss of parietal cells and the resulting reduction in autoantigens. The sensitivity of the IIFT using primate stomach is very high for pernicious anaemia at 80% to 90%. The specificity, however, is limited due to the great variety of other diseases associated with antibodies against parietal cells (e.g. Grave’s disease, Hashimoto’s thyroiditis).

Application of the EUROPLUS™ Stomach (Monkey)/Intrinsic Factor: Indirect immunofluorescence using frozen tissue sections of stomach as the substrate is considered the standard method for the detection of autoantibodies against parietal cells. In positive reactions the cytoplasm of the parietal cells show a fluorescence with fine to coarse plates. The combination of primate stomach and intrinsic factor BIOCHIPs as substrates in IIFT helps to diagnose pernicious anaemia. The prevalence of parietal cell antibodies decreases in the course of pernicious anaemia; many patients develop antibodies against intrinsic factor only much later. Autoantibodies against intrinsic factor can already be detected in some cases of chronic atrophic gastritis (fundus type), even without clinical indications of concurrent pernicious anaemia. The probability that such patients will develop pernicious anaemia later is very high.
Principle of the test: The test system exclusively serves for the in vitro determination of human antibodies in patient serum. The determination can be performed qualitatively or quantitatively. BIOCHIPs coated with the substrate are incubated with diluted patient samples. In the case of positive reactions, specific antibodies of the classes 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.

Autoantibodies against mitochondria (AMA) may lead to incorrect evaluation results. The substrate primate stomach is therefore preincubated with glycine urea buffer to reduce AMA-specific fluorescence.

Test procedure: EUROIMMUN BIOCHIP slides are incubated using the proprietary TITERPLANE Technique. This technique enables multiple samples to be incubated next to each other and simultaneously under identical conditions. Results are evaluated by fluorescence microscopy.

Inter-lot reproducibility: Inter-lot reproducibility was tested with more than 10 different lots. The deviation in the fluorescence intensity of the IIFT amounted to no more than +/− 1 intensity level for all samples.

Reference range: titer 1: < 10

Sensitivity and specificity:

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ig class</th>
<th>Reference (number and origin of samples)</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach (monkey): PCA</td>
<td>IgG</td>
<td>H+/K+-ATPase ELISA (n=126, origin: Germany)</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>EUROPLUS Intrinsic Factor</td>
<td>IgG</td>
<td>Anti-Intrinsic Factor ELISA (n=35, origin: Germany)</td>
<td>—</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Healthy blood donors (n=193, origin: Germany)</td>
<td>99%</td>
<td>—</td>
</tr>
</tbody>
</table>

Technical data:

Antigen substrate: Frozen tissue sections of primate stomach and BIOCHIPs coated with intrinsic factor.

Sample dilution: Serum or plasma, qualitative evaluation: 1:10, quantitative evaluation: 1:10/100/1000 etc. There is no upper limit to the measurement range.

Conjugate: IgG

Test procedure: 30 min (glycine urea buffer) / 30 min (sample) / 30 min (conjugate), room temperature.

Microscopy: Objective 20x, excitation filter: 450-490 nm, colour filter: 510 nm, blocking filter: 515 nm, light source: EUROIMMUN LED, EUROStar Bluelight or mercury vapour lamp, 100 W.

Reagents: Ready for use, with the exception of the PBS Tween buffer.

Stability: 18 months from the date of manufacture at +2 °C to +8 °C.

Test kit format: 10 or 20 slides, each containing 3, 5 or 10 test fields. The kits contain all necessary reagents.

Order number: FA 1362-1005-1 (test kit with 10 slides each with 5 test fields)

Related products: FA 1360-#### (stomach, monkey)
FA 1362-#### (intrinsic factor BIOCHIPs)