Indication: Test system for the in vitro determination of antibodies against proteinase 3 in human serum or plasma for the diagnosis of the following disease: Wegener’s granulomatosis.

Clinical significance: ANCA are autoantibodies directed against antigens found in cytoplasmic granules of neutrophils and monocytes. Several methods are used for the detection of ANCA. Standard technique is the indirect immunofluorescence (IIF) on ethanol-fixed granulocytes. At least two different staining patterns can be differentiated: a granular fluorescence which is distributed regularly over the entire cytoplasm of the granulocytes, leaving the cell nuclei free (cANCA: cytoplasmatic pattern), and a predominantly fine granular fluorescence wrapped around each cell nucleus of the granulocytes (pANCA: perinuclear pattern).

ANCA are typically found in Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA) including renal limited vasculitis, and Churg-Strauss syndrome (CSS), which are all forms of small-vessel vasculitis. These three diseases are grouped together as ANCA-associated vasculitides (AAV) according to the widely accepted classification system introduced by the Chapel Hill Consensus Conference. Classical cANCA are present in most patients with WG (more than 90% in general WG with glomerulonephritis, 70% in limited WG without glomerular involvement) and in about 30% of patients with MPA. Classical cANCA are almost always directed against proteinase 3 (PR3) and very rarely against myeloperoxidase (MPO) or against PR3 and MPO simultaneously. Some cANCA exhibit a flat homogenous cytoplasmic staining in IIF (mostly termed atypical cANCA) which is often directed against bacterial/permeability increasing protein (BPI).

The most important clinical symptoms of ANCA-associated vasculitides are caused by poor blood supply to organs or formation of aneurysms and bleeding due to destruction of blood vessels. Wegener’s granulomatosis is a febrile, chronic granulomatosis disease, mainly of the nasopharynx, lungs and kidney. Since cANCA have been investigated, the diagnosis of Wegener’s granulomatosis has tripled. Due to the high specificity of cANCA the number of diagnosed early-stage and abortive cases of Wegener’s granulomatosis increases steadily.

Application of the Anti-PR3-hn-hr ELISA: The reagent wells of the Anti-PR3-hn-hr ELISA are coated with a mixture of recombinant PR3 (based on human cDNA, expressed in human cells; Sun, Specks et al., 1998) and native PR3. Owing to this, the test has a significantly higher sensitivity (94%) at a very good specificity (98%) compared to other ELISA only using a native antigen (88% and 78%, respectively; study performed in cooperation with the ANCA reference centre University of Maastricht, Prof. Cohen-Tervaert). The International Consensus Statement recommends screening for ANCA using IIF and the confirmation of IIF-positive sera with both Anti-PR3 and Anti-MPO ELISA since the combination of both test systems yields the highest specificity and sensitivity for the diagnosis of small vessel vasculitis.

<table>
<thead>
<tr>
<th>Panels (source: ANCA reference centre University of Maastricht)</th>
<th>m</th>
<th>Anti-PR3-hn-hr ELISA positive</th>
<th>Anti-PR3 Capture ELISA positive</th>
<th>Anti-PR3 ELISA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV (cANCA-positive)</td>
<td>98</td>
<td>55 (56%)</td>
<td>53 (54%)</td>
<td>53 (54%)</td>
</tr>
<tr>
<td>AAV patients</td>
<td>35</td>
<td>33 (94%)</td>
<td>32 (91%)</td>
<td>26 (74%)</td>
</tr>
<tr>
<td>AAV positives</td>
<td>23</td>
<td>23 (100%)</td>
<td>23 (100%)</td>
<td>18 (78%)</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>47</td>
<td>43 (91%)</td>
<td>43 (91%)</td>
<td>36 (77%)</td>
</tr>
<tr>
<td>Sensitivity with respect to IIFT (cANCA)</td>
<td>163</td>
<td>154 (94%)</td>
<td>144 (88%)</td>
<td>127 (78%)</td>
</tr>
</tbody>
</table>

Further autoimmune diagnostics:
circulating immune complexes (CIC) anti-gliadin (IgA, IgG)
Sjögren’s syndrome (IgA, IgM)
ANCA VarioProfile (IgG)
ANA Screen (IgG)
ANA Screen 9 or 1* (IgG)
AMA M2-3E (IgG)
Anti-PR3 ELISA (IgG)
Test characteristics

**Anti-PR3-hn-hr ELISA (IgG)**

**Linearity:** The linearity of the Anti-PR3-hn-hr ELISA (IgG) was determined byassy ing 4 serial dilutions of 6 serum samples. The linear regression was calculated, R² amounting to >0.95 in all samples. The Anti-PR3-hn-hr ELISA (IgG) is linear at least in the tested concentration range of 28 RU/ml to 197 RU/ml.

**Reproducibility:** The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 4 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

**Clinical sensitivity and specificity:** Sera from 163 patients with ANCA-associated vasculitides (AAV; cANCA-positive), a control panel of 585 patients with other diseases and 429 healthy blood donors were analysed using the EUROIMMUN Anti-PR3-hn-hr ELISA (IgG). The sensitivity of ELISA for cANCA-positive AAV was 94%, at a specificity of 99%.

**Reference range:** Levels of anti-PR3 antibodies were investigated in 429 sera from healthy blood donors between 19 and 68 years of age (172 women, 257 men) using the EUROIMMUN ELISA. No differences with respect to age or gender were observed. The mean concentration of antibodies against PR3 was 2.2 RU/ml (± 9.6 RU/ml of standard deviation) and the values ranged from 0.1 to 171.7 RU/ml. With a cut-off of 20 RU/ml, 4 blood donors were anti-PR3 positive.

**ROC analysis:** In an analysis of 140 samples from patients with ANCA-associated vasculitides (cANCA-positive) and 1014 control samples the following characteristics were determined:

**Technical data:**

**Antigen:** Mixture of native proteinase 3 from human neutrophils and recombinant proteinase 3, based on human cDNA and expressed in human cells.

**Calibration:** Quantitative, in relative units per milliliter (RU/ml).

- **Calibration serum 1:** 200 RU/ml
- **Calibration serum 2:** 20 RU/ml, cut-off
- **Calibration serum 3:** 2 RU/ml

**Sample dilution:** Serum or plasma; 1:101 in sample buffer.

**Reagents:** Ready for use. Exception: wash buffer (10x). Colour-coded solutions, largely exchangeable with those of other EUROIMMUN ELISA.

**Test procedure:** 30 min / 30 min / 15 min. Room temperature. Fully automatable.

**Measurement:** 450 nm. Reference wavelength between 620 nm and 650 nm.

**Kit format:** 96 single break-off wells, incl. all necessary reagents.

**Order no.:** EA 1201-9601-2 G
Important information for users of the EUROIMMUN Anti-PR3-hn-hr ELISA

The Anti-PR3-hn-hr ELISA is a new milestone in the serological diagnosis of Wegener’s granulomatosis. The antigen substrate used consists of two components: human native proteinase 3 (PR3) and recombinant PR3 expressed in a human cell line (source: Institute for Experimental Immunology, a facility of EUROIMMUN AG). The already potent recombinant PR3 is supplemented with native antigen in order to be able to present the complete antigen spectrum, since the recombinant antigen does not always possess all native antigen epitopes.

Special characteristics of the recombinant antigen component

- Human cells are used for the first time worldwide for the expression of PR3 in full-scale production. In human systems the posttranslational modifications that take place are authentic and true to species, in contrast to (heterological) insect cells or bacteria usually used. Therefore, the target antigen used for diagnostics conforms better to the natural one.
- In recombinant PR3 the proteolytic active centre is artificially switched off by the exchange of an amino acid.
  - Since the proteinase activity of the enzyme no longer interferes with cell metabolism, the cultured cells can accumulate PR3 in high concentrations – without this manipulation they die early.
  - The synthesised PR3 does not digest itself in an uncontrolled manner in the preparation and can be produced in large quantities.

Comparison of results

The new Anti-PR3-hn-hr ELISA is much more sensitive than conventional Anti-PR3 tests: in a panel from Damoiseaux et al. (Ann Rheum Dis, 2008 Mar 28, Epub ahead of print) a sensitivity of 95% was obtained for the Anti-PR3-hn-hr ELISA (conventional Anti-PR3 ELISA: 80%), with respect to the indirect immunofluorescence test and calculated for a specificity of 99%.

Comparison of results

<table>
<thead>
<tr>
<th>Serum</th>
<th>Measurement values for the concentration of Anti-PR3 AAb (IgG)</th>
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<tbody>
<tr>
<td></td>
<td>Conventional Anti-PR3 ELISA</td>
</tr>
<tr>
<td>1</td>
<td>8 RU/ml</td>
</tr>
<tr>
<td>2</td>
<td>22 RU/ml</td>
</tr>
<tr>
<td>3</td>
<td>64 RU/ml</td>
</tr>
<tr>
<td>4</td>
<td>196 RU/ml</td>
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</table>

Four examples

Consequences for the laboratory daily routine

If conventional Anti-PR3 tests are replaced by the new Anti-PR3-hn-hr ELISA, it must be expected that:
- some previously negative sera react positively (example 1),
- higher measurement values are generally obtained for antibody concentrations (sometimes many times higher, examples 2 and 3),
- in individual cases comparable values are obtained (example 4),
- measurement values do not always increase by the same ratio; the percentage increase in values can vary substantially from serum to serum.

We recommend that you inform your customers about the change of test in writing. We are happy to provide data sheets for this purpose. If you wish you can use the following text, in whole or in part: “We will in the future be using a new test system for the determination of antibodies against PR3 [EUROIMMUN Anti-PR3-hn-hr ELISA], which detects up to 15% more cases of Wegener’s granulomatosis or ANCA-associated vasculitis. With this test a purely technically caused increase in measurement values for antibody concentrations can occur, which should not be interpreted as an increase in clinical activity. The increase can vary in strength from case to case”.

EUROIMMUN AG · Seekamp 31 · 23560 Lübeck · Tel 0451/58 55-0 · Fax 58 55-591 · E-mail euroimmun@euroimmun.de · www.euroimmun.de

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