

Test Instructions IMTEC-PCA-Antibodies (cut-off)

Enzyme Immunoassay for the Detection of Anti-Parietal cell Antibodies

REF : TC 30800

Please read the instructions carefully before testing.

Procedural precautions:

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different lots.
- ▶ Store reagents at 2-8°C.

1. Clinical Use

Antibodies to Parietal cell antigens (PCA) were described with patients with a pernicious anaemia (Addison's anaemia) for the first time. The gastric H⁺/K⁺-ATPase was identified as the targeted autoantigen.

In earlier studies PCA antibodies were detectable in approx. 90% of patients with a pernicious anaemia. Only 55% of these patients, however, showed PCA antibodies in a younger study. The differences might originate from the different human races of the mentioned studies.

Furthermore PCA antibodies were described in patients with autoimmune endocrinal diseases like Hashimoto Thyreoiditis and diabetes mellitus type I (20-30%) as well as chronically atrophied gastritis type A.

Though PCA antibodies have no absolute specificity, since they are also detectable in healthy people (5-10%) and their prevalence is increasing with advancing age too.

2. Principle of the Test

The test is based on the immobilisation of highly purified H⁺/K⁺-ATPase to the solid phase of microtitre plates (polystyrene), and subsequent binding of PCA antibodies. For the detection of PCA antibodies bound to the microtitre plate an antibody directed to human IgG, conjugated with peroxidase, is used. After addition of peroxidase substrate solution, a color stain develops, its intensity is proportional to the concentration and/or the avidity of the PCA antibodies.

3. Materials Provided

- MTP : H ⁺ /K ⁺ -ATPase coated microtiter strips (1x8), breakable	12 strips
- CONTROL co : cut-off control, ready to use, contains sodium azide	1 vial 2 ml
- CONTROL - : negative control serum, ready to use, contains sodium azide	1 vial 1 ml
- CONTROL + : positive control serum, ready to use, contains sodium azide	1 vial 1 ml
- BUF WASH 10x : washing buffer concentrate (10x)	1 bottle 50 ml
- DIL SPE 5x : sample buffer concentrate (5x)	1 bottle 22 ml
- CONJ a(hum IgG):HRP : HRP-Conjugate, anti-human IgG, ready to use	1 bottle 12 ml
- SUBS TMB : TMB solution, HRP substrate, ready to use	1 bottle 12 ml
- SOLN STOP : stopping solution, ready to use, contains sulfuric acid, caution corrosive!	1 bottle 12 ml

4. Preparation of Reagents

Attention! Allow the testkit and all its components to reach room temperature completely before executing it!

Please do not use any polystyrene vessels for handling of HRP conjugates.

If the test is performed automatically, we recommend the use of fresh conjugate each run and to discharge traces of old conjugate entirely. Remove washing buffer after washing steps completely.

4.1. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part washing buffer concentrate **BUF** **WASH** **10x** with 9 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

4.2. Preparation of Sample Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part sample buffer concentrate [DIL] [SPE] [5x] with 4 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2-8°C.

4.3. Control Sera, HRP Conjugate, Stopping Solution and TMB Solution

Control sera, HRP conjugate, stopping solution and TMB solution are ready to use. Used bottles should be closed carefully and stored at 2-8°C. **Store TMB solution also protected from light.**

4.4. Preparation of Sera

Use serum samples freshly collected or freeze samples at -20 °C. Allow sera to reach room temperature (30 min). Dilute sera 1 : 100 with sample buffer (10 µl sample to 1 ml buffer).

4.5. Microtiter Strips

The strips are ready to use. Unused strips should be sealed and stored in the lockable original bag at 2-8°C.

5. Test Procedure

- **Pipette 100 µL serum dilution** or control sera [CONTROL] [+], [CONTROL] [co] and [CONTROL] [-] into each well, for blank use sample buffer instead of serum dilution, seal wells with adhesive foil.
- **Incubate for 1 hour** at room temperature (RT).
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µL of HRP-conjugate** [CONJ] [a(hum IgG):HRP] into each well, seal wells with adhesive foil.
- **Incubate for 30 minutes** at RT.
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µL TMB solution** [SUBS] [TMB] into each well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- **Pipette 100 µL stopping solution** [SOLN] [STOP] per well.
- **Measure at 450 nm** within the next 30 min after stopping.

6. Interpretation of Results

To prove the functionality of the test, the absorbance of the positive control serum [CONTROL] [+] has to be distinctly higher than the absorbance of the cut-off control [CONTROL] [co]. The absorbance of the negative control [CONTROL] [-] serum has to be lower than the cut-off control [CONTROL] [co] of the test.

A patient serum with a measured absorbance that is distinctly higher than the absorbance of the cut-off control [CONTROL] [co] possesses an enhanced level of specific antibodies (positive).

Precautions

For in vitro diagnostic use only.

[IVD]

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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