ECP ELISA

ELISA for the quantitative determination of the eosinophilic cationic protein (ECP) in human serum

Eosinophilic granulocytes are involved in different inflammatory processes, including atopic, parasitic and rheumatic diseases. The most prominent feature of the eosinophils are their large secondary granules, each containing four basic proteins, the best known protein is the eosinophil cationic protein (ECP). Elevated ECP levels are found in atopic diseases such as allergic asthma and allergic rhinitis but also occasionally in other diseases such as bacterial sinusitis.

The ECP level in humans correlates with the disease activity or the severity of diseases of the atopic disorders. For this reason the parameter provides externalization and therapy monitoring of bronchial asthma or atopic dermatitis.

The ECP ELISA for the quantitative measurement of ECP in human serum is based on a Sandwich ELISA. During the first incubation step ECP from the patient sample is captured by an anti-ECP-antibody coated to the microwells. By a washing procedure surplus serum components are removed from the well. During the next incubation step a biotinylated anti-ECP-antibody is added and incubated in the microwells. After a further washing step the detection is carried out with a streptavidin/peroxidase (HRP)-conjugate forming complexes consisting of ECP / biotinylated anti-ECP-antibody / HRP-conjugate. The wells are washed again, and the substrate solution 3,3‘,5,5‘-Tetra-Methyl-Benzidine (TMB) is added and incubated, resulting in the development of a blue colour.

ECP Specifications

- Test duration ≈ 3h (60min/ 60min/ 30min/ 30min)
- 20µL Serum or Plasma per determination
- Detection: HRP/TMB
- 6-point calibration
  (0; 2,0; 6,0; 20; 60; 200 ng/mL)
- Large measurement range (until 200 ng/mL)
- Limit of detection 0,5 ng/mL

ECP REF 25070 Σ 96

After stopping the enzymatic reaction with acid the colour changes into yellow. The optical density (OD) of the coloured product is measured spectrophotometrically at 450 nm (reference wave length 620 nm)Calibrators with defined concentrations of ECP are assayed simultaneously with the patient samples to generate a calibration curve. Unknown ECP concentrations of the test samples are calculated from this curve.

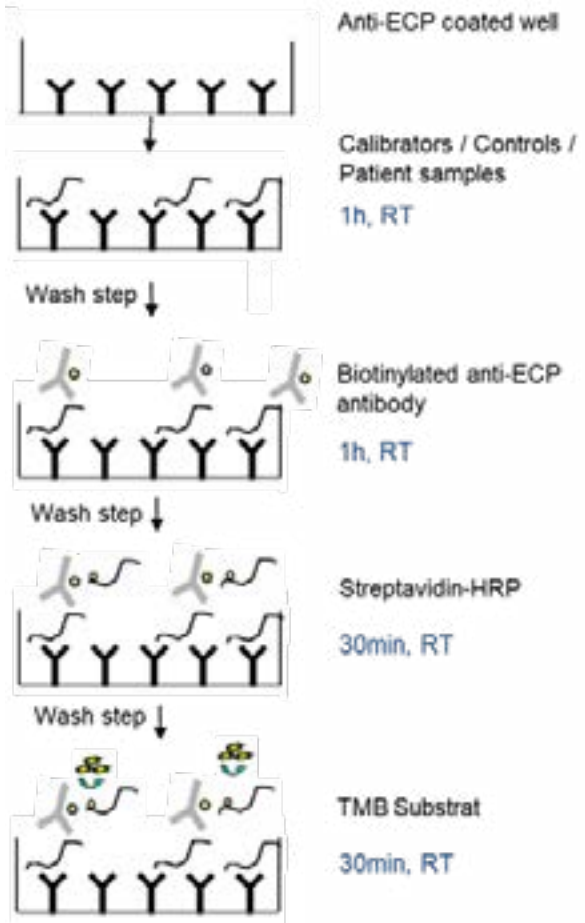

Figure 1:
Principle ECP Test

Dr. Fooke-Achterrath Laboratorien GmbH - Habichtweg 16 - 41468 Neuss - Germany
Phone: + 49 2131 2984-0 - Fax: + 49 2131 2984-184 - email: information@fooke-labs.de

Performance Data

The intra-assay, inter-assay and lot-to-lot variations of the test were found within the following specifications:

Intra-Assay Variation	< 10%
Inter-Assay Variation	< 15%
Inter-Batch Variation	< 20%

In healthy individuals the ECP concentrations have been described between 1-15 ng/mL. Every person shows his own baseline level which usually stays constant over the time. An increased ECP concentration, approx. > 10 ng/mL, can be an indication of an allergic inflammation for example bronchial asthma or atopic dermatitis.

A linear behaviour up to 150 ng/mL of the ECP ELISA is confirmed. It is not mandatory that values >150 ng/mL are within a linear behaviour because of the saturation of the calibrator curve in this area.
The results of the assay have always to be considered in the context of the clinical situation of the patient and his anamnesis.

In an evaluation, the overall sensitivity and specificity (compared to a commercial available ECP ELISA) were determined as 100% and 88%, respectively (Poster DGAKI, 2020).

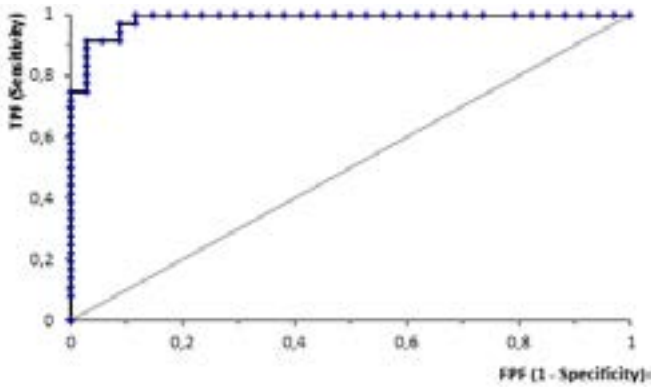

Figure 2: ROC analysis for ECP ELISA (Dr. Fooke Laboratorien GmbH) vs. a commercial available ECP ELISA with n=70 results.

Spearman correlation between ECP ELISA from Dr. Fooke Laboratorien and a commercial available ECP ELISA reveals a correlation coefficient of 0.98 (CI 0.97-0.99).

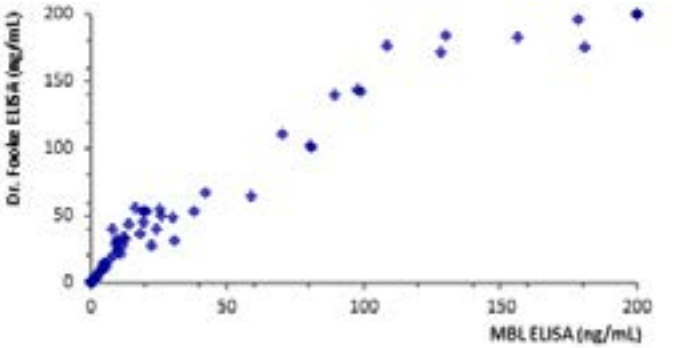

Figure 3: Spearman correlation between ECP ELISA from Dr. Fooke Laboratorien GmbH and a commercial available ECP ELISA with n=70 results.

Literature

1. Bystrom J, Amin K, & Bishop-Bailey D (2011): Analysing the eosinophil cationic protein-a clue to the function of the eosinophil granulocyte. Respir Res 12:10.
2. Niimi A & Matsumoto H (1999): Serum measurement of eosinophil cationic protein in the management of asthma. Curr Opin Pulm Med 5(2):111-117.
3. Escudero Pastor A & Hernandez Garcia J (1993) [Diagnostic performance of eosinophil protein quantification. Review]. Allergol Immunopathol (Madr) 21(6):233-240.
4. Czerwionka-Szaflarska M & Gasiorowska J (2006) [Eosinophil cationic protein as a marker of eosinophil activity]. Przegl Lek 63(7):579-582.
5. Kapp A (1993) [The role of eosinophilic granulocytes for the pathogenesis of atopic dermatitis /neurodermatitis. Eosinophilic products as markers of disease activity]. Hautarzt 44(7):432-436.

DR FOOKE

Dr. Fooke-Achterrath Laboratorien GmbH - Habichtweg 16 - 41468 Neuss - Germany
Phone: + 49 2131 2984-0 - Fax: + 49 2131 2984-184 - email: information@fooke-labs.de