

Development of an enzyme-linked immunosorbent assay kit for determination of the major allergen from *Dermatophagoides pteronyssinus*, Der p 1, house dust mite

Kees van der Graaf, Oswin ten Brinke, Sjoerd Blok, Citeq Biologics, Groningen
Wim van Oeveren, Haemoscan, Groningen, Henk Bak, Eurosequence, Groningen

Introduction

House dust mite allergy is one of the most wide spread allergic diseases. Most patients allergic to house dust mite react to Der p 1 from *Dermatophagoides pteronyssinus*, which has been shown to be a cysteine protease.

Monoclonal and polyclonal antibodies are important in biochemistry, biology and pharmacy. Antibodies can be used for crude extract standardization, allergens purification, quantification of environmental allergens, epitopes mapping of allergens, and immunochemistry.

Two-site enzyme-linked immunosorbent assay (ELISA) for groups 1 and 2 dust mite allergens have been widely used for environmental allergens quantification and allergen standardization.

Currently there are no reliable ready-to-use-kits for the determination of Der p 1. Therefore we developed a ready-to-use-ELISA for Der p 1.

Approach

A ninety-six-wells plate (NUNC Maxisorp) was coated with a monoclonal antibody (clone 9-5-1) against Der p 1 generated in a mouse. The monoclonal-antibody was obtained from a hybrid between BALB/c spleen cells and a myeloma cell line.

Samples and a standard are incubated for one hour at room temperature. For the standard we used a purified standard obtained from Der p 1 whole culture material.

A rabbit-polyclonal antibody (from rabbits immunized with purified Der p 1) was used and incubated for 30 minutes, followed by a peroxidase-labeled detection antibody and substrate for detection.

Afterwards cleavage of the substrate was quantified by measuring the optical density at 450 nm using a microplate reader.

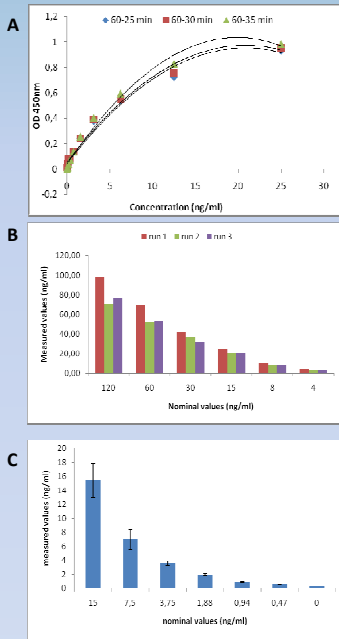
According to EMA guidelines CPMP/ICH/381/95 and performed according to Analytical Method Validation conform ICH (Nov, 2005)

Contents of the ELISA kit

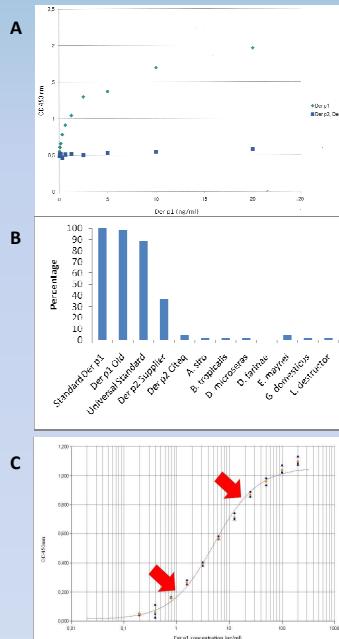
- Pre-Coated NUNC 96-wells plate in zip closed pouch
- 2 ml Sample dilution buffer 20x
- 500 µl Standard Der p1 (0.1% sodium azide)
- 300 µl Quality control low (0.1% sodium azide)
- 300 µl Quality control high (0.1% sodium azide)
- 15 ml Wash Buffer 30x
- 1 ml Dilution buffer A (Red) 10x (0.1% sodium azide)
- Der p1 polyclonal antibody A
- 1 ml Dilution buffer B (Green) 10x
- PO labeled detection antibody B
- 11 ml Substrate
- 11 ml Stop solution

Results

Robustness (A)/Repeatability (B, C)

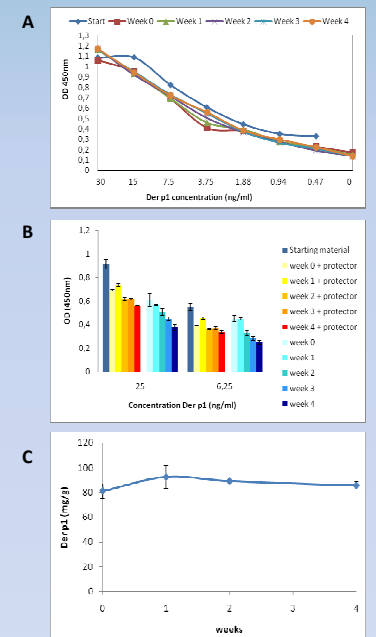


Specificity (A, B)/Sensitivity (C)



Stability

Capture antibody (A), GaR-HRP (B), standard (C)



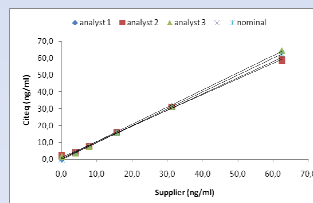
Conclusion

- A highly sensitive and specific two-site ELISA has been developed.
- The assay system developed is useful for the quantification of Der p 1 in mite extracts and environmental dust extracts
- The working range of the Der p 1 ELISA is between 1 to 15 ng/ml
- The CV's of the accuracy and precision were below 15%
- Robustness/repeatability, specificity/sensitivity, and stability show good results for each of these variables
- All of these variables were tested according the EMEA guidelines for validation and according to Analytical Method Validation conform ICH
- There is a small degree of cross-reactivity with *E. maynei* (< 5%)

Comparison of Citeq Der p1 ELISA kit with another Der p1 ELISA is shown below

No clear differences are observed

Therefore, the advantage is, our Der p1 ELISA kit is reliable, ready-to-use and time saving



Der p 1 ELISA kit

The kit comes with a Certificate of Analysis



Info: elisa@citeq.nl