Smart Optical Detection of Food Allergens using CD technology

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Aim

The objective of this research is to develop a highly sensitive and selective fast, cheap and reliable method for detection of major Food Allergens; Gluten (***Gliadin***), Milk Proteins (***Casein and β-Lactoglobulin BLG***), Egg protein (***Ovalbumin***) and Soybean Protein (***Trypsin Inhibitor***) simultaneously with only **ONE Compact Disk** utilizing Sandwich Immunoassay technique.

Key Words **ELISA:** enzyme-linked immunosorbent assay **MAB:** Monoclonal Antibody, **HRP**: Horse Radish Protein

Introduction

Food allergies are increasing in prevalence [1]. It is estimated that about 5% of young children and 3% to 4% of adults have food allergies [2]. Among the major allergens believed to be responsible for up to 90% of food allergies, milk and egg allergens are the most prominent. Due to its simplicity, sensitivity, and accuracy for native proteins, the most common method used for allergen detection is (***ELISA***). Food allergy denotes an immunologic mechanism represented almost exclusively by ***IgE-mediated*** reactions [3]. Allergic symptoms, (Fig.1), are varied from eczema, asthma, rhinitis, vomiting, diarrhea, cramps, till ***anaphylactic shock*** and in some cases may lead to death [4,5].

Fig (1): Food Allergy symptoms

Method

The ***Sandwich ELISA*** technique (Fig.2) is adopted on compact disk surface which act as a platform for immobilization of (**MABs**), which allow using nano-quantities, printed on the CD by Aspirate/Dispense Platform (***BioDot AD1500)*** and provides a large area for printing several antibodies. Each ***MAB*** react with the corresponding Allergen protein, then adding **MAB** conjugated with **HRP** which later detected by **TMB** and then take readings. (Fig.3)

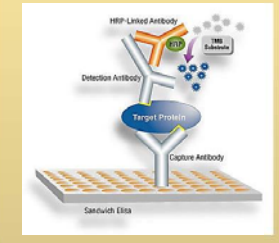


Fig (2): Sandwich ELISA: The target protein is sandwiched between two MABs one of them is HRP conjugated then detected by the change of TMB color by addition of H_2O_2

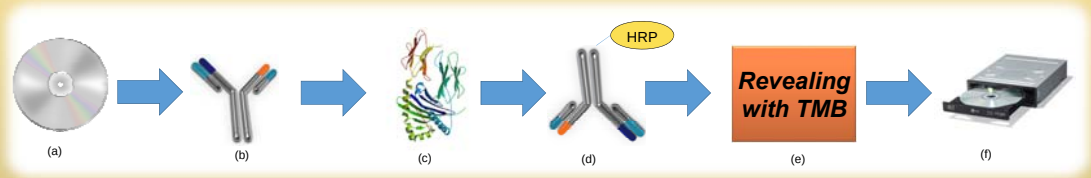
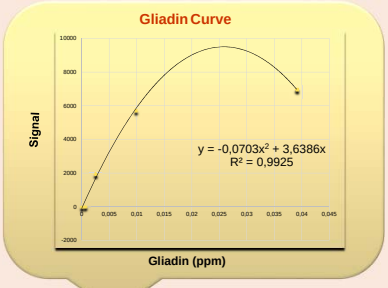
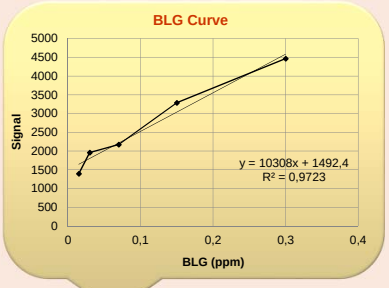


Fig (3): a,b) Printing CD with the MAB Diluted in Carbonate buffer pH 9.2 and incubate Over night in Room temp. **c)** Adding samples or the target protein standard diluted in PBST and incubated for 30 min. R.T. **d)** After washing with PBST and distilled H2O, Adding Ab.-Conjugate diluted in PBST and incubate for 30 min. R.T. **e)** wash then reveal with TMB (dark incubation for 10 – 20 min.). **f)** Wash with dist. H2O and take readings by CD Drive. *(a customized software is utilized for data reading and analysis is used)*

Results

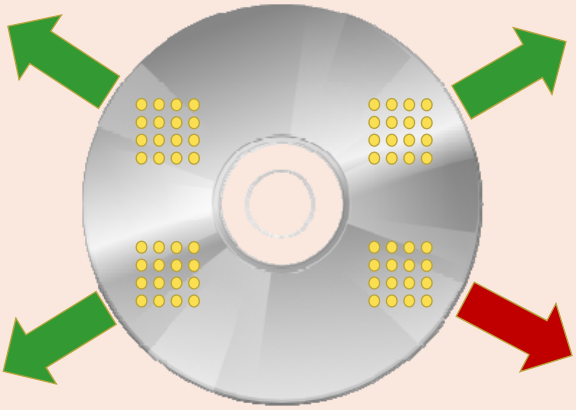


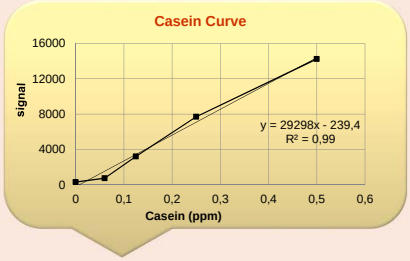
LOQ = 2.44 ppb
LOD = 1.2 ppb
R^2 = 0.9925



LOQ = 0.17 ppm
LOD = 0.12 ppm
R^2 = 0.9723

LOQ: limit of quantification
LOD: limit of detection





LOQ = 0.062 ppm
LOD = 0.031 ppm
R^2 = 0.99

Allergens Under investigation
Trypsin Inhibitor and Ovalbumin

Future work

1- Completing our set of the four selected allergens to be detected simultaneously by one CD.
2- Development of a unique extraction protocol for the 4 food allergens ***(Gluten, Milk, Egg, and Soy)***

Acknowledgments

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References

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