# ANTIBODY FUNCTIONALIZED GOLD NANOPARTICLES FOR MULTIPLEX DETERMINATION OF FOOD ALLERGENS

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## Abstract

Around 2% of the population and up to 8% of children suffer from food allergy, with symptoms ranging from relatively mild to severe, or sometimes even fatal consequences[1]. In commercial processed food products, allergenic foods are used as ingredients in many food products for their nutritional value. European Directive 2003/89/EC reinforces the general rule that all substances that have been intentionally introduced in food stuff should be indicated under their specific name in the list of ingredients[2]. For that, an effective analytical technique is necessary for ensuring food safety for allergic people. We present a multiplex competitive microimmunoassay on a compact disc for testing food allergens under the labeling regulations. The assay is developed for the simultaneous determination of wheat (gluten), milk (casein and β-Lactoglobulin) and egg (ovalbumin) proteins using specific antibody functionalized gold nanoparticles. The detection limit achieved was 0.18, 0.03, 0.03 and 0.09 mg/L for gluten, casein, β-Lactoglobulin and ovalbumin respectively. The assay was evaluated by the analysis of both liquid and solid spiked food samples, reaching recovery values ranging from 72-112%, demonstrating its suitability for the simultaneous determination of allergens in less than 60 minutes.


## Experiment

### Labeling antibodies

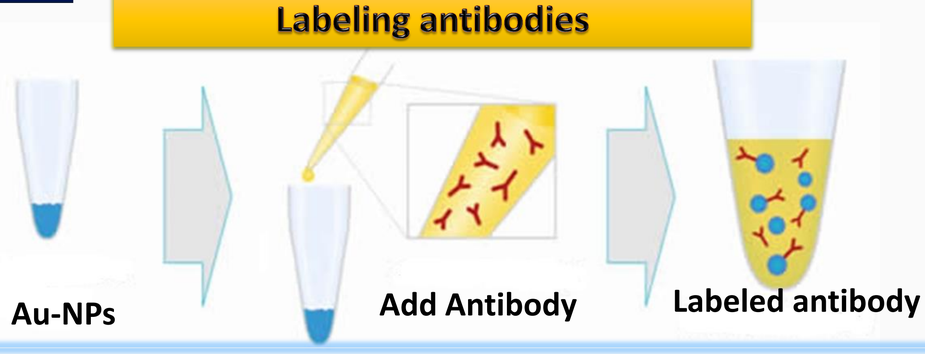



*Fig.1*

- Au-NPs pH were adjusted to 8.5 using drops of 0.05M carbonate buffer.
- 50 µg of Ab. was added to 1 mL of AUNPs suspension/ stirrer/30 min.
- 100 µl of 10% BSA-Tris base 20 mM, pH8.5/stirrer/30 min. Centrifuge 1hr/15000 rpm/4 °C.
- Supernatant discarded and the pellet was resuspended with 1 mL Tris 20 mM, pH8.5.
- Centrifuge 1hr/15000 rpm/4 °C.
- Supernatant discarded and the pellet was resuspended with 100 µl 1% BSA-Tris 20 mM, pH8.5

### Protein ammobilization on CDs

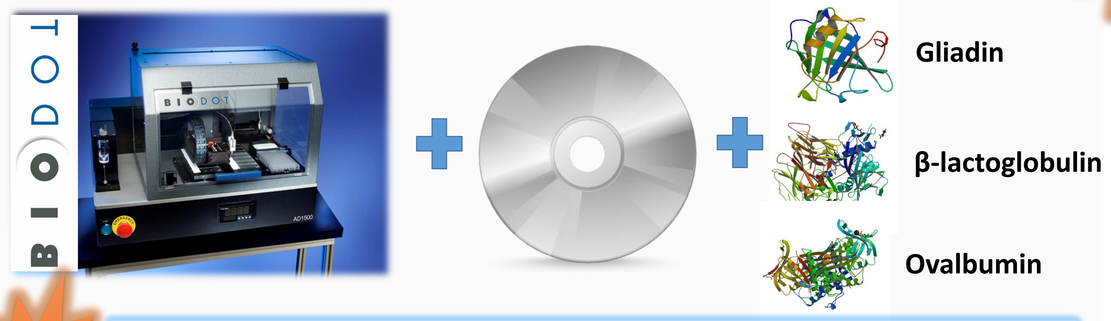



*Fig.2*

- Proteins were diluted in Carbonate buffer to a concentration of 5, 10, 10 and 20 ppm for gliadin, casein, β-lactoglobulin and ovalbumin respectively.
- Microarraying machine, BIODOT AD1500, was used for printing CDs with the proteins.
- CDs were incubated over night at room temperature and ready to be used from the next day.

### How does it work ?

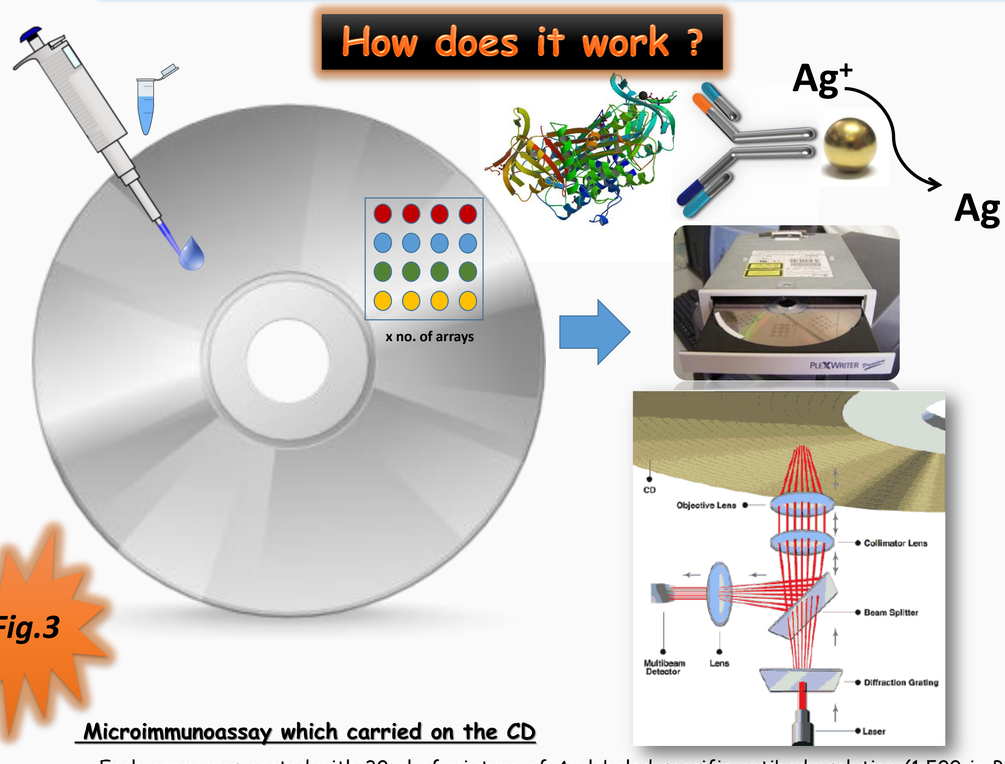



*Fig.3*

**Microimmunoassay which carried on the CD**

- Each array was coated with 30 µl of mixture of Au-labeled specific antibody solution (1:500 in PBS-T) containing extracted sample or analyte.
- After 30 min, the CD was washed with PBS-T and dist. $H_2O$.
- In order to display the immunoreaction, arrays were incubated with 1 ml of 1:1 (v/v) silver enhancer solution, and the reaction was stopped after 8 min by washing with water.
- After drying, the result were read using CD drive.
- Reflected laser beam from the CD due to immunoreaction products can be measured by specific software (**BioDisc**) and translated to signals.

## Results

### Optimization of each allergen assay individually

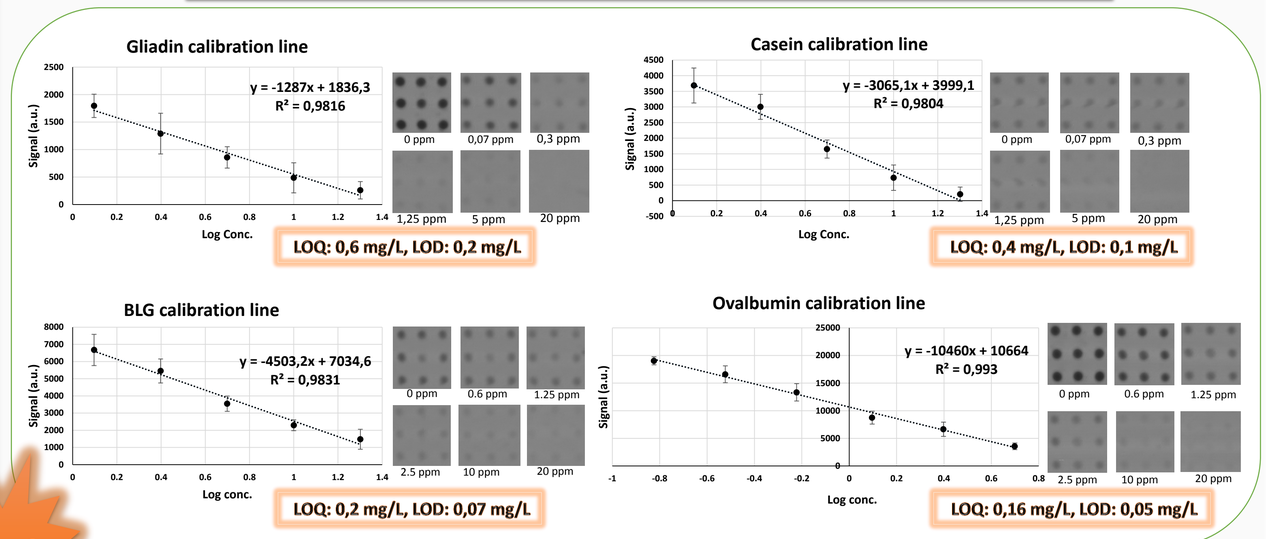
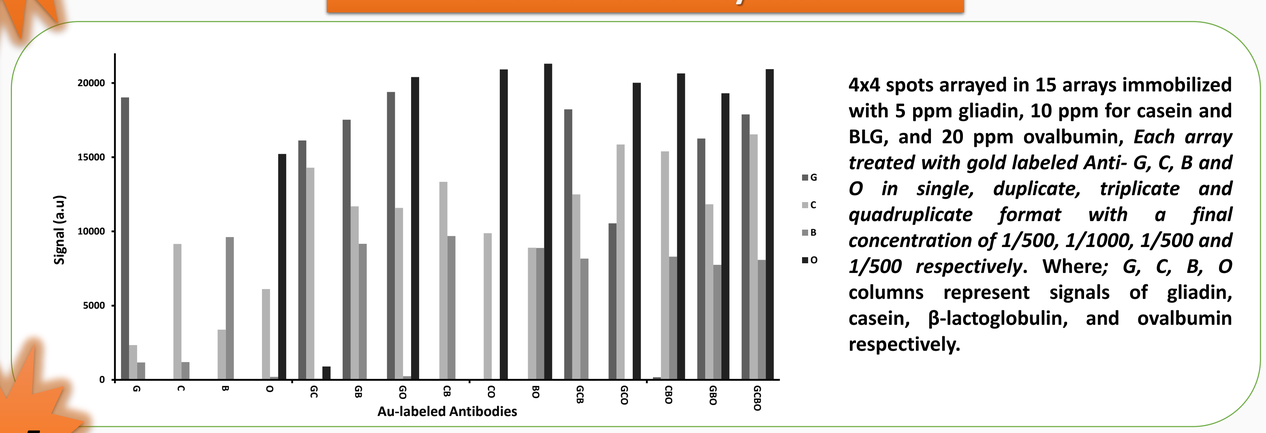



*Fig.4*

### Shared reactivity test




4x4 spots arrayed in 15 arrays immobilized with 5 ppm gliadin, 10 ppm for casein and BLG, and 20 ppm ovalbumin, *Each array treated with gold labeled Anti- G, C, B and O in single, duplicate, triplicate and quadruplicate format with a final concentration of 1/500, 1/1000, 1/500 and 1/500 respectively*. Where*; G, C, B, O* columns represent signals of gliadin, casein, β-lactoglobulin, and ovalbumin respectively.

*Fig.5*

### Optimization of simultaneous detection of the 4 allergen

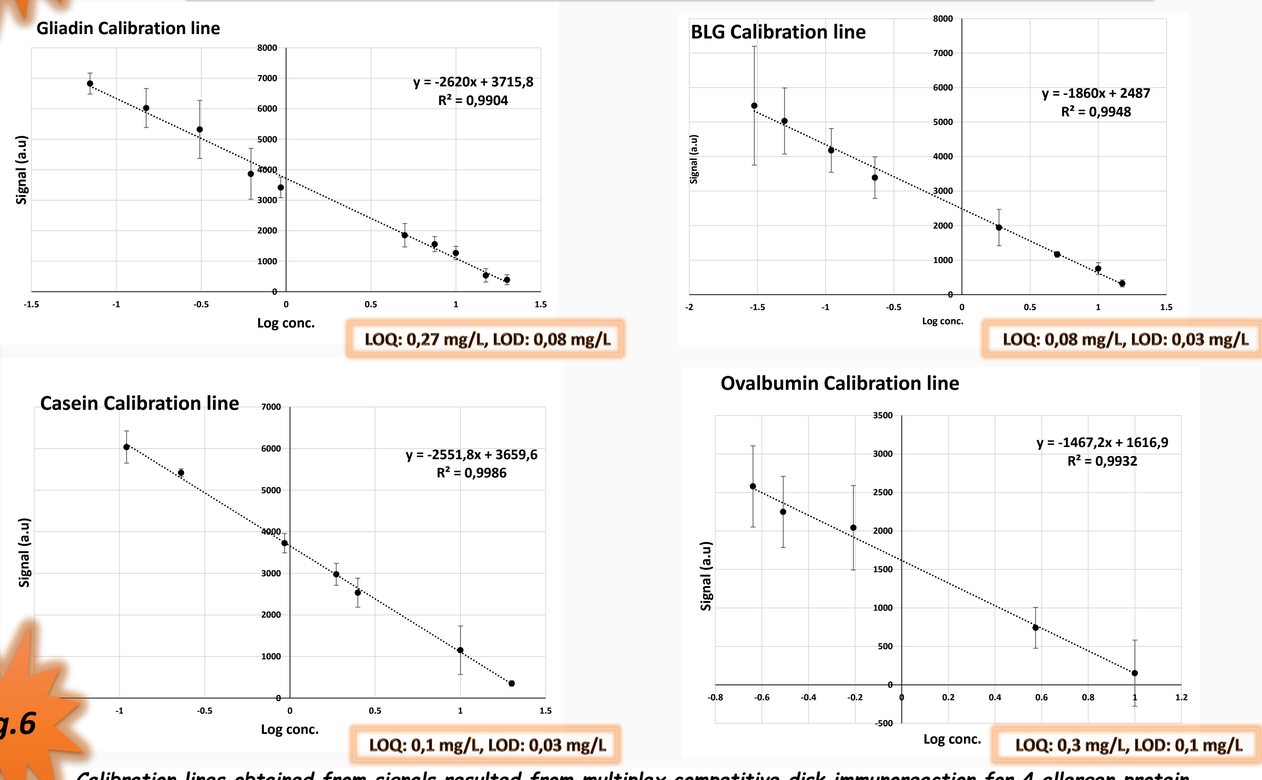



*Fig.6*

*Calibration lines obtained from signals resulted from multiplex competitive disk immunoreaction for 4 allergen protein. LOQ: limit of quantification, LOD: limit of detection*
**a), b), c) and d) are calibration curves for G, C, B and O respectively. e) Signal Intensity is inversely proportional to allergen concentration in the assay**

## Future work

- Currently an extraction protocol is being optimized.
- Different food samples from the market are to be analyzed.
- Environmental allergens should be assayed using the same technique..

## Acknowledgments

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## References

1. Sampson, H. A. Allergy, 2005, 60, 19-24., 2. European Commission. Directive of the European Parliament and of the Council of 10 November 2003 amending Directive 2001/13/EC. Official Journal, L308, 15, 25 Nov. 2003.