



Setting standards
in analytical science



Government Chemist Dissemination Event 2008

LGC – Food Allergens Method Validation

Indu Patel

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The Problems



- Relatively few published validated methods
- International consensus on validation protocols for ELISA and DNA allergen methods remains in development.
- The miss-identification of an allergen in food could be fatal for an allergic consumer as there is no cure
- Have devastating consequences for food businesses.



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Tree Nut - Allergy



- Data is limited but In the United States and in the United Kingdom the prevalence of tree nut allergy is estimated to range between 0.2% and 0.5% both in children and in adults
- It is also known that tree nuts, are one of the most frequent causes of food allergy.
- Test kits for Peanut allergen have been extensively validated by AOAC
- We therefore decided to validate a test method for walnut which can cause allergenic response by airborne pollen and severe food allergy.



Walnut



- *Juglandaceae* family
- Contain 60 species
- Nuts from all species are edible
- Persian Walnut (*Juglans regia*) is widely cultivated
- Also known as common/
- English or European walnut
- Has larger nut and is easy to crack



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Validation Process

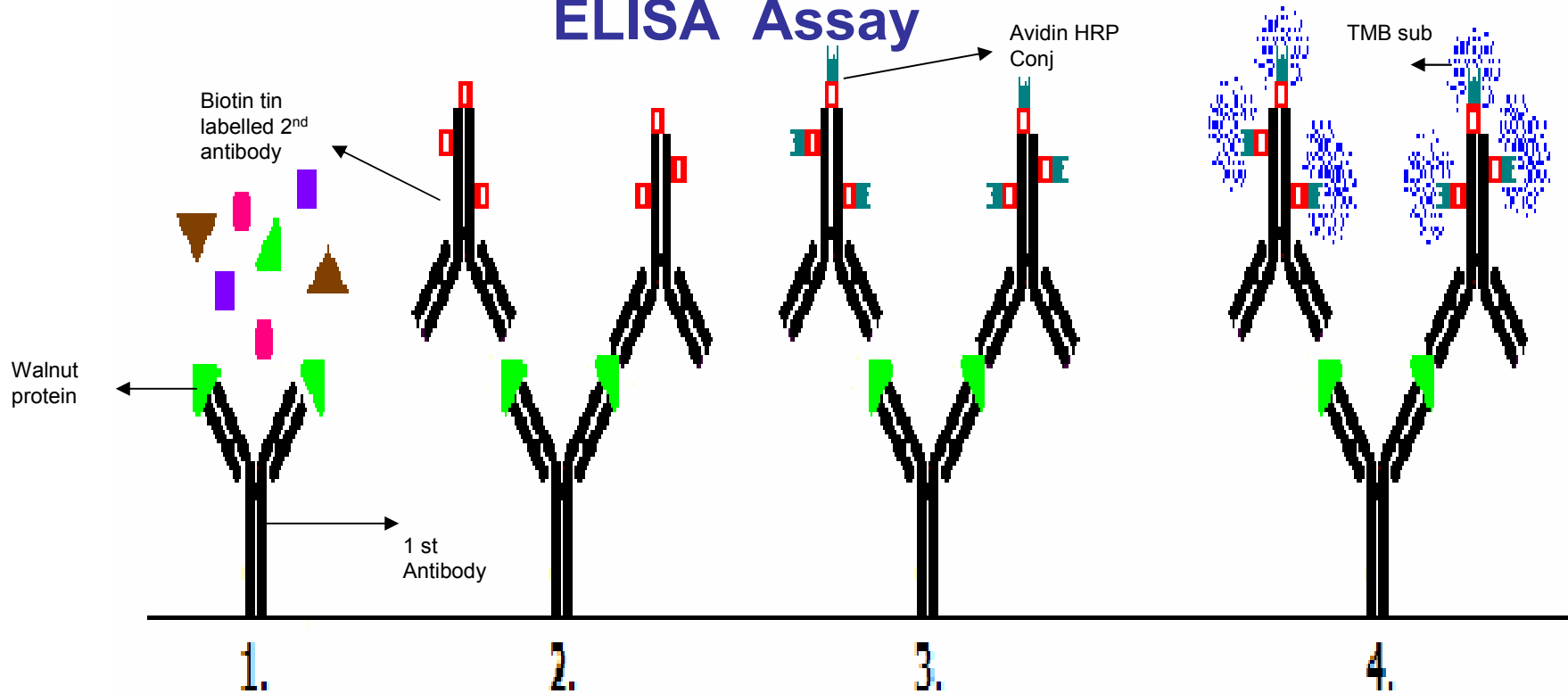


- Broadly based on AOAC
- LOD
- Cross reactivity/Interference
- Sensitivity/Specificity
- Robustness



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ELISA Assay



- 1) Walnut protein (Δ) in food binds to antibody on well.
- 2) Biotin labelled 2nd antibody binds to walnut protein bound to antibody on well.
- 3) Avidin –HRP enzyme conjugate binds to biotin labelled antibody.
- 4) Addition of TMB develops blue colour in presence of enzyme. Addition of stop solution changes colour from blue to yellow.



- We used pre-commercialised ELISA kit - kindly supplied to us on our request

Protein Contents of Walnuts By LECO

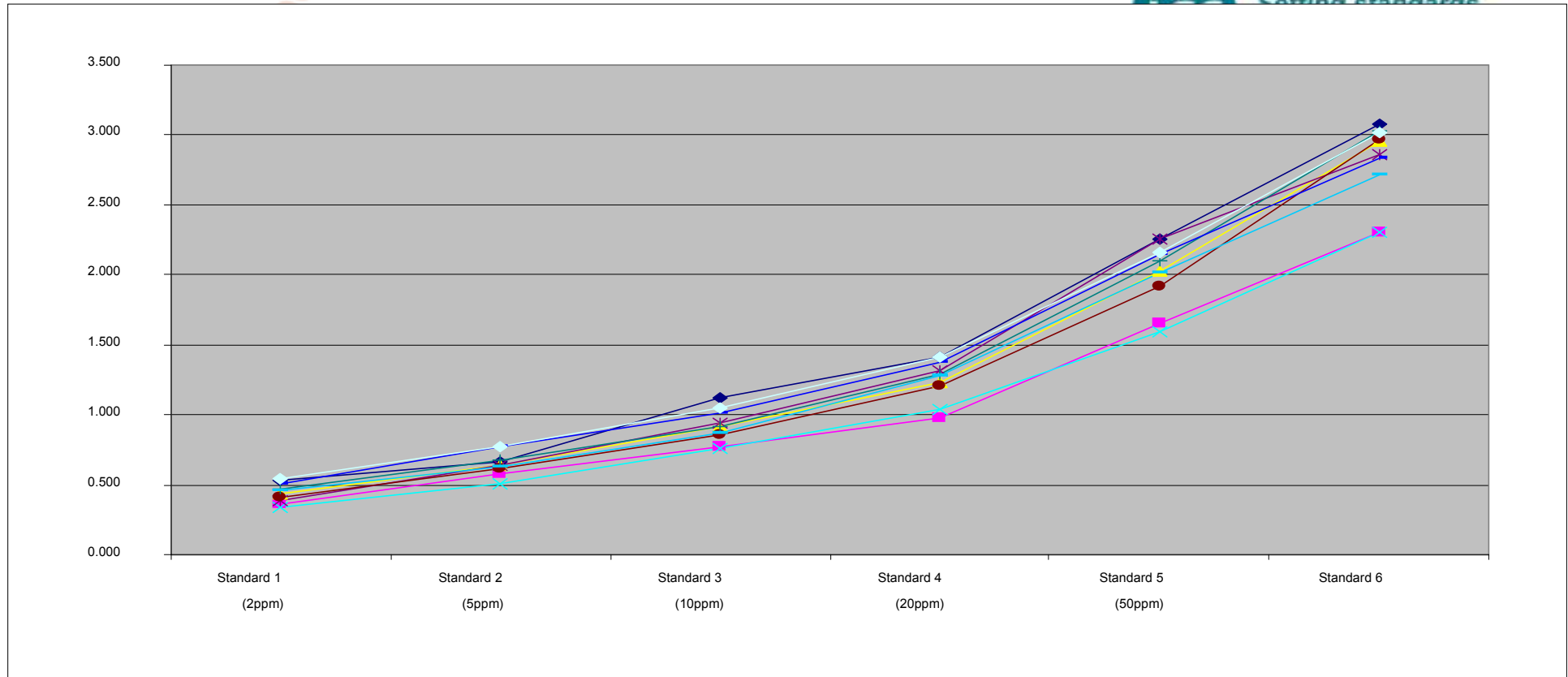


- Moldovan walnuts : 16.34%
- California walnuts : 15.09%
- Walnuts from France : 13.20%
- Walnuts from Chile : 14.19%
- Walnuts from more than one country : 16.03%

All walnut proteins may not be allergenic



Walnut Protein Kit Standards - LOD



- The LOD calculated using data from ten separate assays by interpolating the walnut protein concentration from the mean of the Reagent Blank Optical Density (OD) + 3 x Standard Deviations (SD) of standard 0.
- The overall LOD using all the data from the ten assays was found to be 0.811 pap, i.e. below 1 pap.



Food Sample Matrices/ Interference



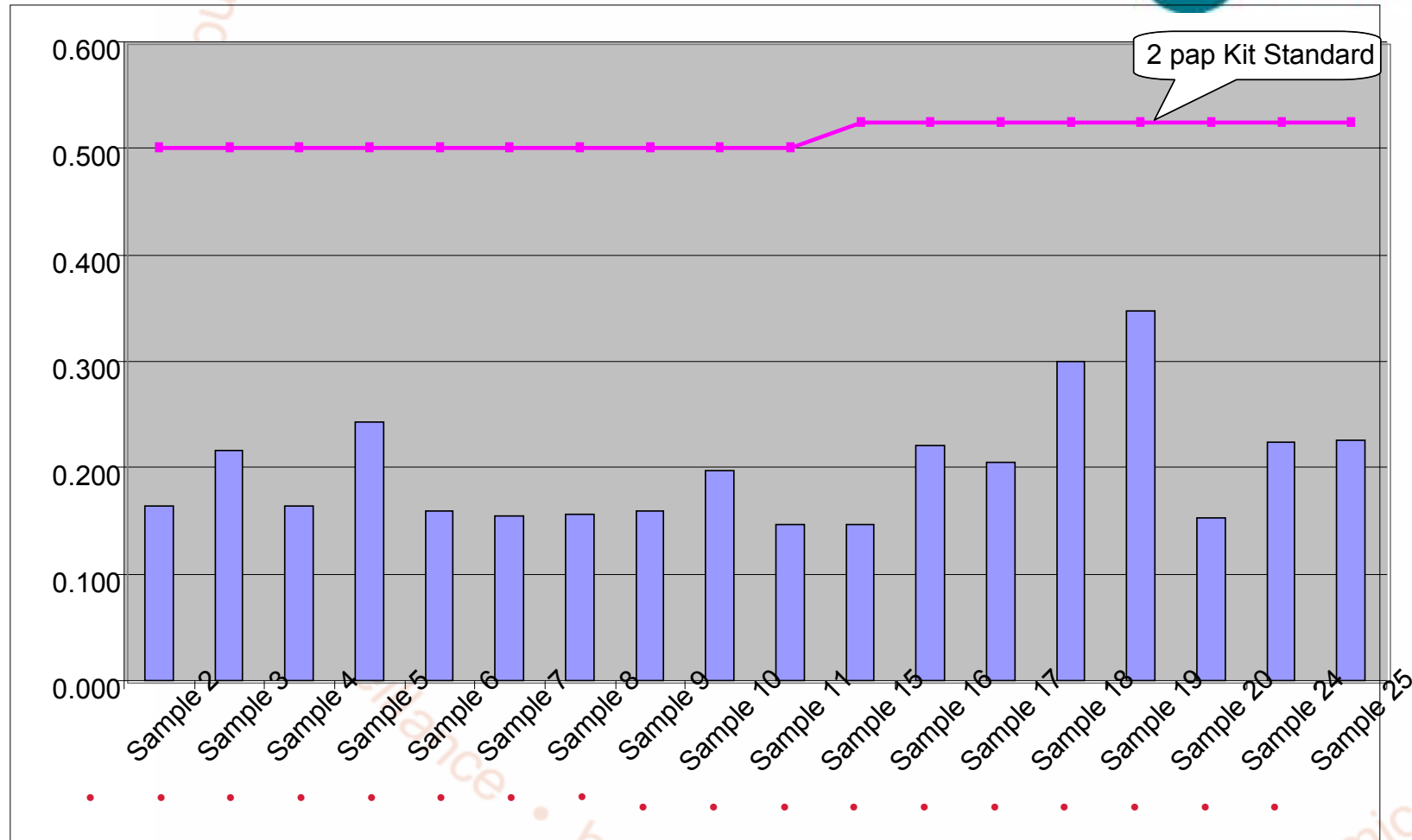
Food Matrices with Walnut Declared



- Correctly identified 9 sample commodities purchased from local supermarkets which listed walnut as one of ingredients.
- Walnut protein was not detected in one sample of crunchy bar in which it was the last ingredient on the list. It is possible that walnut content of crunchy bar is < 1ppm or bulk material is inhomogeneous .



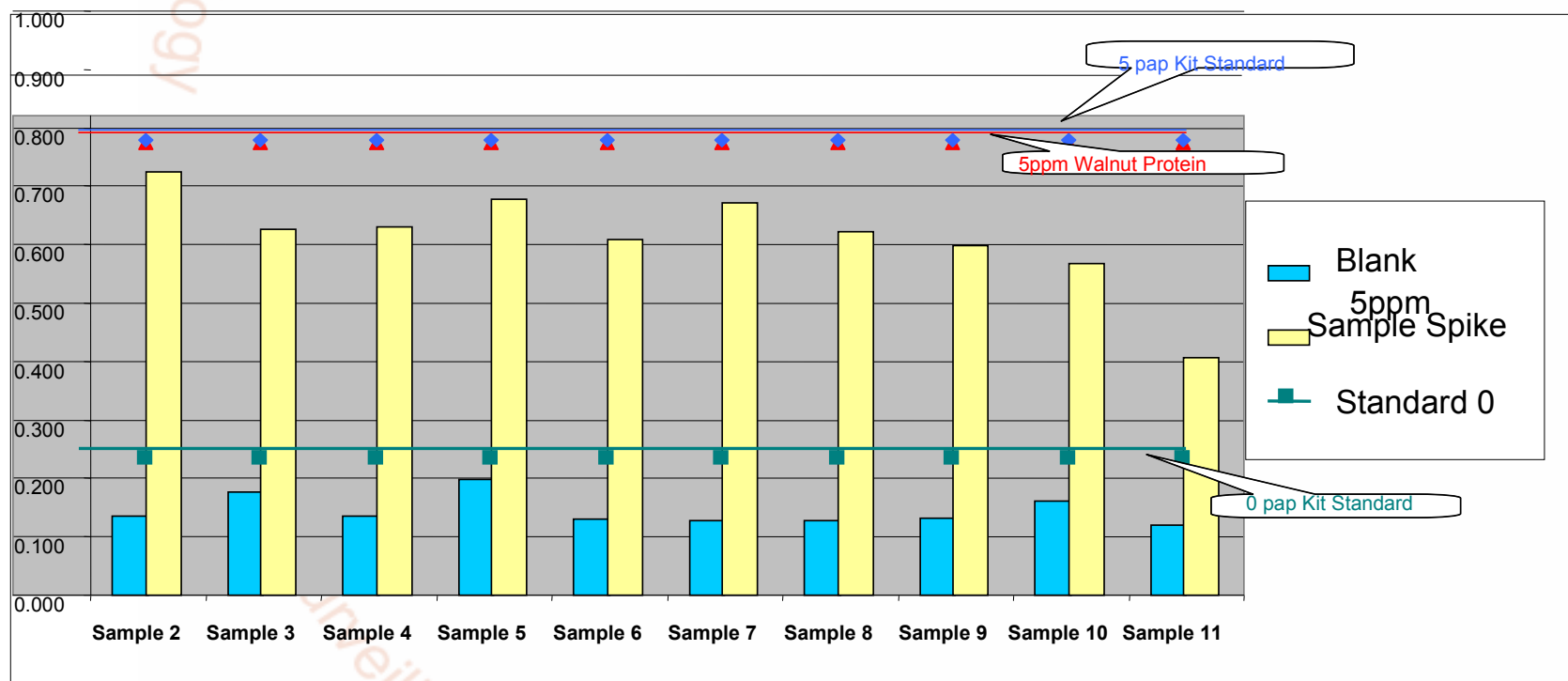
Food Matrices with no Walnut



Correctly identified 100% of the sample commodities which, did not list walnut as one of the ingredients on the retail pack, OD values of all samples were below 2 pap kit standard.



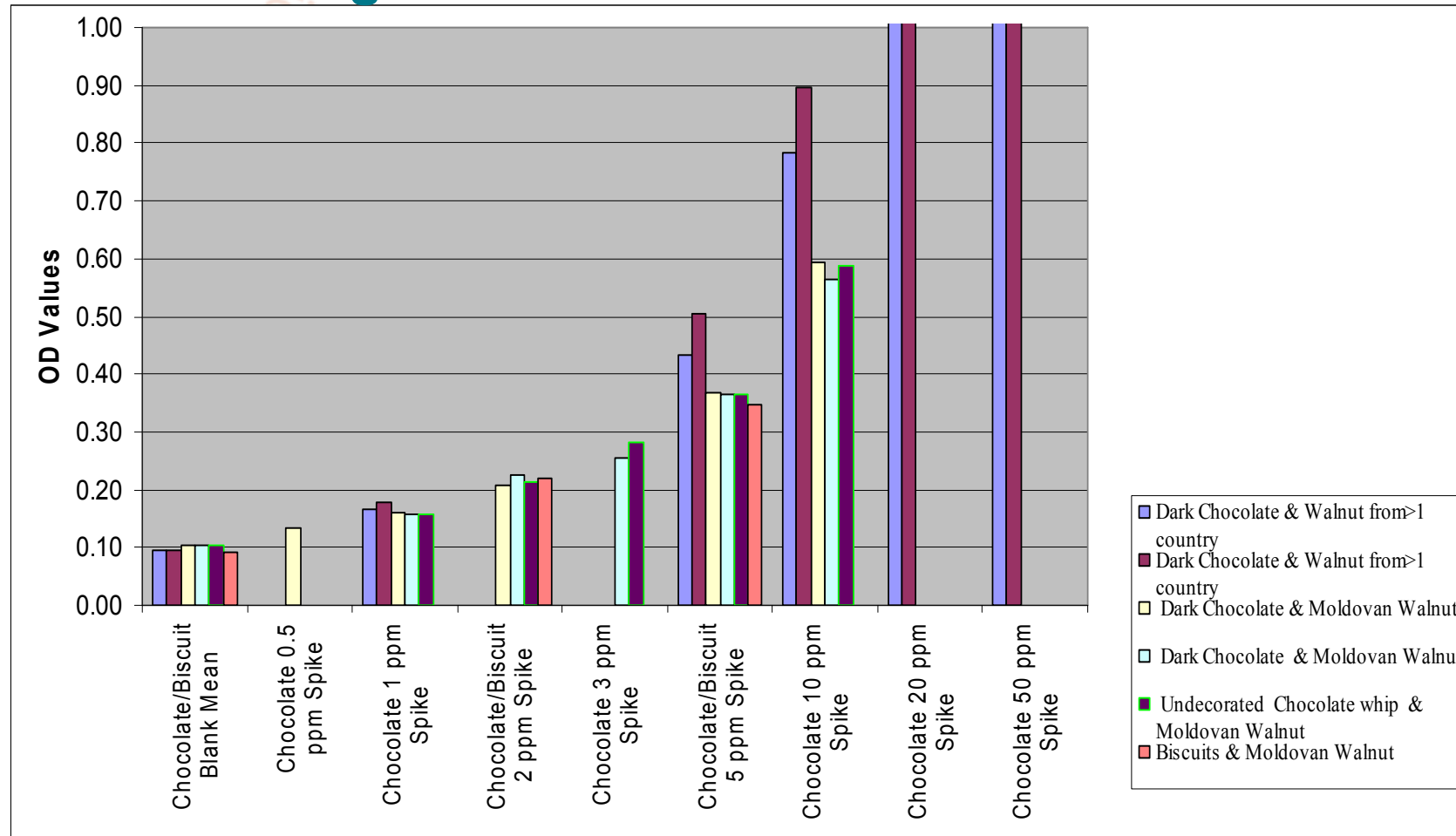
Recovery checks



- Satisfactory recoveries (89 – 130%) were obtained from all the spiked processed food matrices including biscuits, cakes, cereal and chocolate bars
- A sample of *Options chocolate drink* gave a low recovery at 38 %.



Further Investigation with Chocolate matrices



- **Chocolate is an acknowledged difficult matrix.**
- **Investigated a variety of chocolate and biscuit samples in our evaluation including Dark Chocolate, Milk Chocolate with Cream & Biscuit**
- **Walnut - Walnut from more than one Country**
- **- Walnut from Moldova**



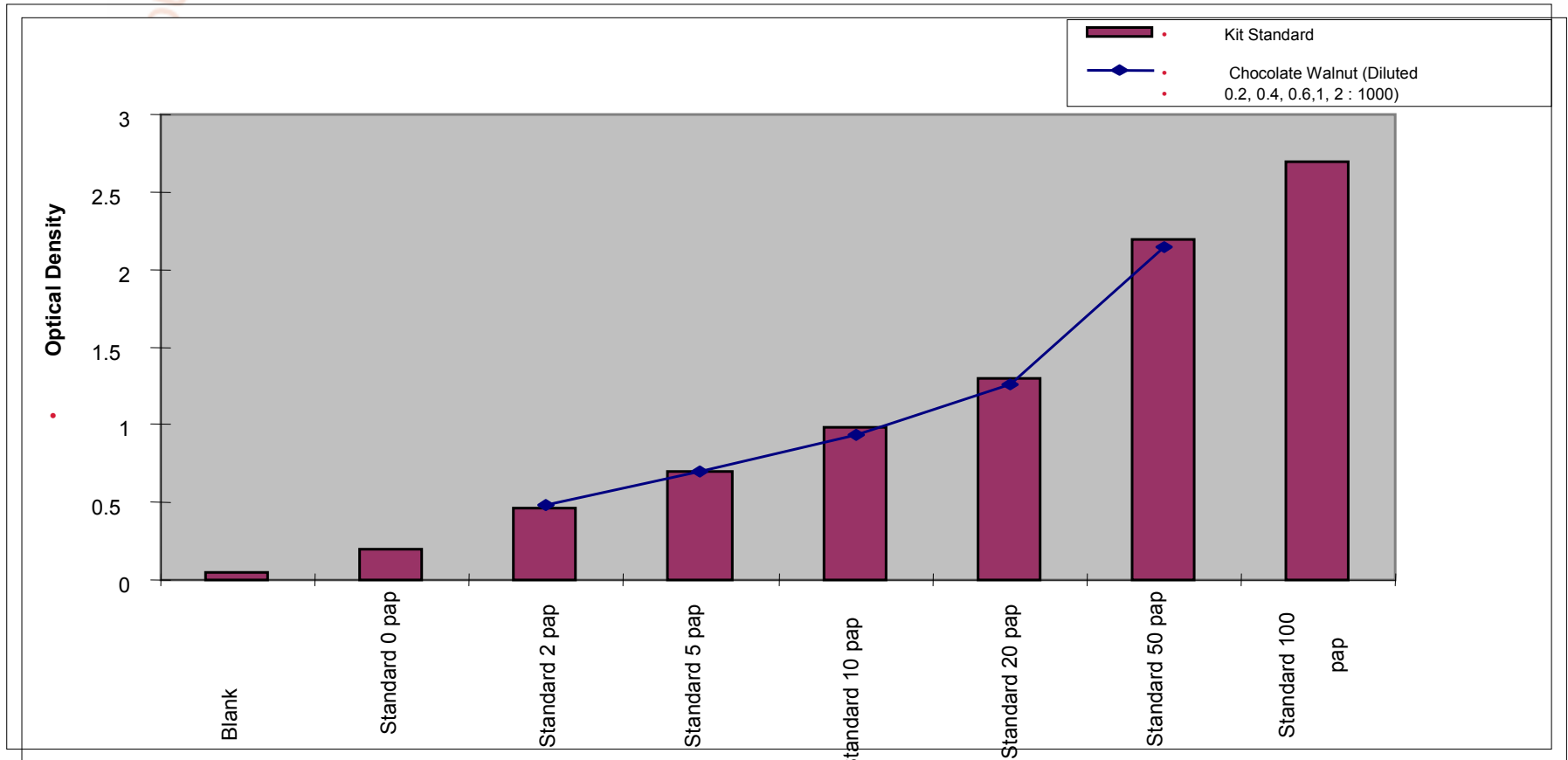
Investigation of Cross-reactivity/Interference with Other Ingredients



- 35 commodities including
- Nuts, Grains, Proteins, Seeds
- Slight Cross Reactivity with Poppy seeds and Pecan
- All other commodities gave results below the detection limit of 1 ppb



Linearity and Storage Stability



A sample of chocolate/walnut was diluted at five levels to correlate with walnut protein standards provided in the assay kit. An appropriate linear response was demonstrated with diluted sample extracts of a chocolate/walnut confectionary



Robustness -



Extraction Variables

- Sample Weight
- Extraction Volume
- Extraction buffer composition
- Extraction buffer temperature
- Extraction Time
- Extraction motion
- Extract dilution



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ELISA Variables

- Sample reagent volume
- Laboratory temperature
- Incubation times
- Washing
- Time after incubation
- Addition Drift
- Incubation



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Conclusion



- Validation exercise was successful
- The method can be reliably used to detect 1 pap walnut protein in food products
- However it is important to run a blank QC and spiked control of a similar matrix to the sample for testing to assess interference and cross reactivity
- We will publish our results within next few months



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Segregation at LGC to Minimise Allergen Cross Contamination



- Known positive Lab 5/12
- Blank Sample Prep Lab 5/13
- Known Unknown Sample Prep Lab 5/1 - White coats
- Dilution of Extracts Lab 5/24 - Blue coats
- Restricted access to trained staff only
- No Entry if nut consumed on the day
- Protective clothing worn in lab area
- Disposable lab ware used for sample preparation
- Dedicated Homogenisers used



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Allergen Laboratory – Sample Preparation Laboratory 5/24



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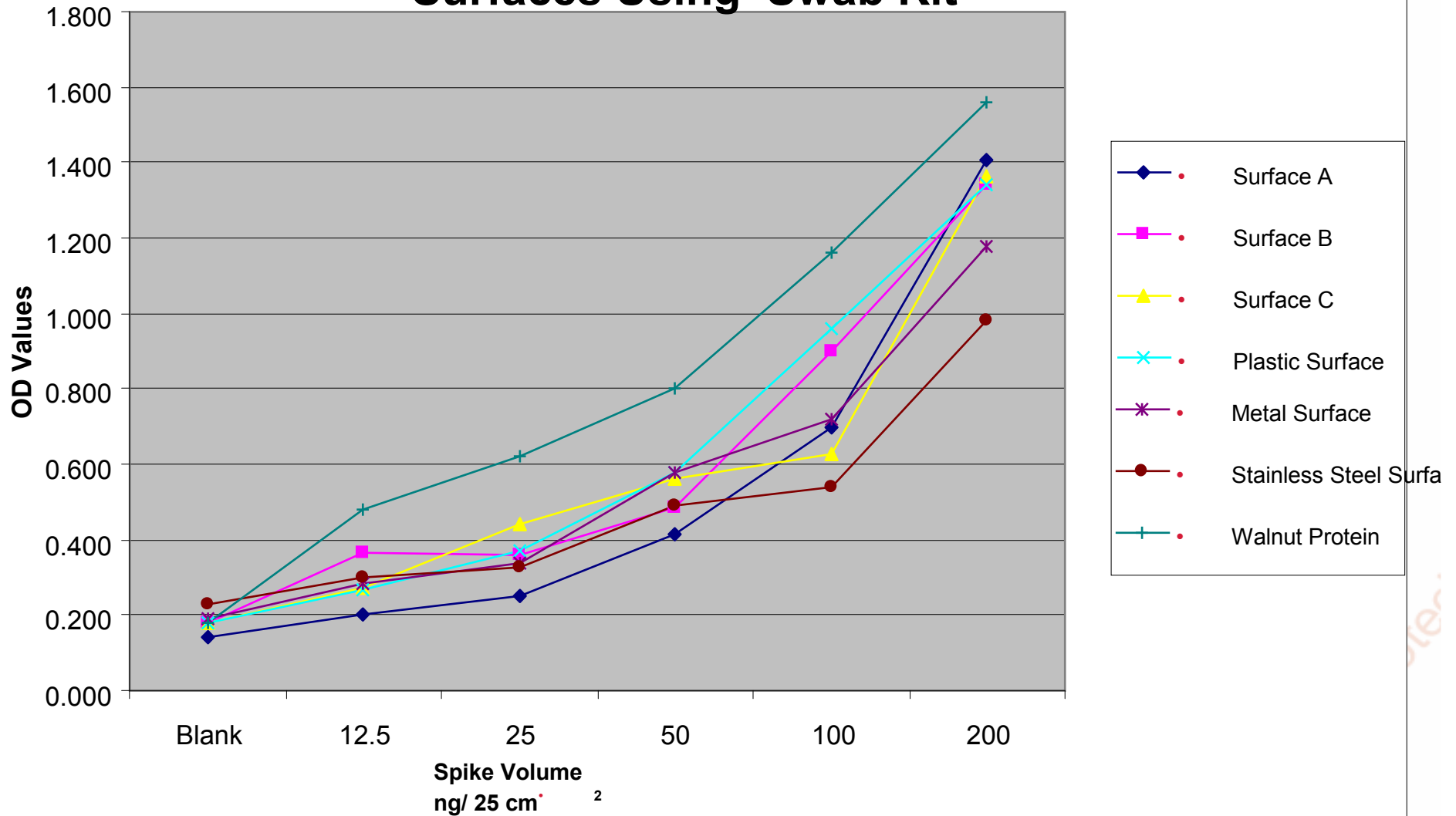
Allergen Laboratory – ELISA Process Laboratory 5/1



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Evaluation of Swab Kit To Monitor Contamination Comparison of Walnut Protein Transfers from Different Surfaces Using Swab Kit



Contamination of walnut protein at a concentration of above 30 ng/ in 25 cm² area of can be detected from all 6 surfaces

Investigation of Other Allergen Kits



- Peanut - Police forensic case study is still under a dispute
- Almond kit – Evaluated after spiking with chocolate and Biscuit matrix at 1 pap level.
- Evaluation of other allergen kits
 - Soya
 - Egg
 - Gluten
 - Milk



Finally Thanks



- Malvinder Singh for helping to validate this procedure
- LGC for major funding and facilitating the validation
- DIUS for partially funding the analytical work in this study



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