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Towards the development of a dipstick immunoassay for the detection of trace amounts of egg proteins in food

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Abstract Immunoassays using antigen-specific antibodies offer a powerful tool for the detection of the analyte proteins in a complex food protein mixture. This study reports the first approach to the development of a dipstick assay for the determination of egg proteins in food. Dipstick tests are very inexpensive, rapid, and portable, and do not require technical skill to perform. The polyclonal antibodies specific to egg proteins, developed in Health Canada, that do not cross-react with any of the 38 nuts, legumes or other common food ingredients tested will be used to develop the dipstick test for egg. Different food samples were tested. A non-competitive format with direct spotting of the antibody onto a nitrocellulose membrane and detection with an antibody coupled to peroxidase was used. The assay proved to be sensitive enough to detect trace amounts of egg protein in the lower microgram per kilogram range.

Keywords Egg allergens · Dipstick · Immunoassay · Cross-reactions

Introduction

Food allergy is a distinct class of adverse reaction to food which involves abnormal immunological responses i.e. IgE-mediated responses to certain food proteins in sensitive subjects. It is estimated that about 2% of all people show allergic reactions when they consume cer-

tain foods [1]. Among the group of allergenic foodstuffs, peanut, milk, seafood, soya, tree nuts, egg, fish, shellfish and gluten can cause severe allergic reactions [2, 3]. Allergy against egg, for example, can be caused by relatively small amounts of egg proteins and can exhibit typical symptoms like skin rash, coughing, vomiting, and/or difficulty in breathing [4]. Life-threatening anaphylactic reactions to eggs are very rare. Unlike peanut allergy, children generally outgrow their egg hypersensitivity. For the detection of allergenic protein residues from food products, e.g. egg proteins in noodles [5], it is necessary to develop specific and sensitive analytical methods since trace amounts of allergic proteins can cause allergic symptoms. The method of choice is the enzyme-immunoassay [6, 7, 8]. In this work the first approach to the realization of a dipstick assay for the determination of trace amounts of egg proteins in food is described.

Materials and methods

Immunization of rabbits with ovalbumin, purification of the sera, and characterization of the antibodies was carried out at the Laboratory of Dr. Yeung according to [9].

Extraction of egg protein for the preparation of a standard. Yolk and egg white from boiled eggs were separated. The yolks were extracted three times with 200 ml hexane. Egg white and defatted egg yolks were combined and the proteins were extracted with 100 ml phosphate buffered saline (PBS), (0.01 M K_2HPO_4 , 0.15 M NaCl, pH 7.4) for 1 h, centrifuged (10,000×g, 30 min) and stored frozen in 1 ml aliquots.

Sample extraction. The sample (10 g) was soaked with 100 ml extraction buffer (1% Tween 20, 0.4% Triton X100 in PBS buffer, pH 7.4) for 30 min, crushed for 30 s with a homogenizer, extracted for 30 min (100 rpm on a rotary shaker), filtered through glass wool and centrifuged for 30 min at 10,000×g at 4 °C. The protein content was determined according to Bradford with bovine serum albumin (BSA) as standard [10].

Coupling of antibodies to horseradish peroxidase. Horseradish peroxidase (HRP) (10 mg) was mixed with 0.2 ml of 1% glutaraldehyde solution and stored overnight at room temperature. After a clean up with a PD-10 column (Pharmacia) the brown fraction was collected and 5 mg antibody in 1 ml PBS buffer and 0.2 ml of

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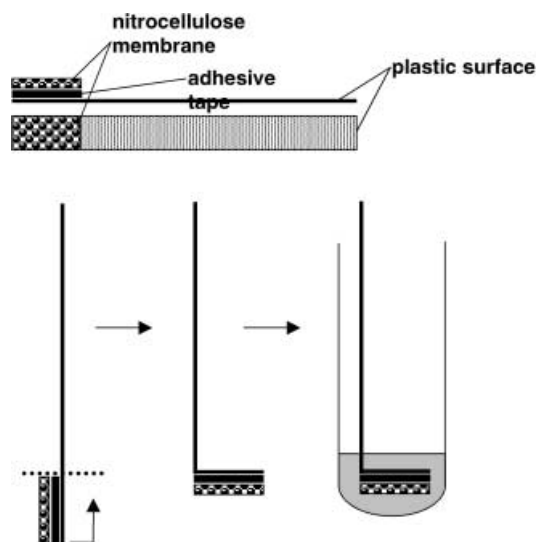


Fig. 1 Dipstick assembly: the nitrocellulose membrane is pasted to the plastic stripe with an adhesive tape. The part of the plastic with the nitrocellulose is folded to minimize the volume of the antibody solution

0.1 M carbonate-bicarbonate buffer were added. After 18–24 h at 4 °C the reaction was stopped with 0.2 ml of 1 M glycine. The solution was centrifuged with Centricons (Amicon, 100 kD), and afterwards diluted with glycerine (equal volume) and stored at –20 °C.

Immunoassay. Nitrocellulose was cut into 5 mm stripes and mounted on a plastic surface (Fig. 1). 1 µl of the of anti-egg white antibody solution (~0.98 µg protein, 1:300) was spotted on a nitrocellulose membrane with a Hamilton syringe, dried for 10–15 min and than blocked with 3% milk powder in PBS. The membrane was washed three times with 0.1% Tween 20 in PBS. After drying the membrane was incubated with the sample solution for 30 min. The membrane was washed again as mentioned above. Afterwards the membrane was incubated with the anti-egg white-HRP antibodies for 30 min. The staining reaction was performed according to Vieths et al. [11] employing tetramethylbenzidine and diocetyl-sodium sulphosuccinate, which yields stable blue precipitates.

Results and discussion

Three antibodies were available for the test development. All three antibodies were coupled to HRP. To avoid cross reactivities and false positive results, all antibodies were incubated with HRP antibodies (Table 1).

The whole-egg antibody showed cross-reactions in all combinations. Only the antibodies against egg white and ovalbumin could be used. Further experiments with egg standard showed that the ovalbumin antibody was less

Table 2 Determination of the limit of detection for the dipstick assay, using butter biscuits and garlic dressing. Pos Positive, neg negative

Spiked egg standard µg/kg	Repetitions	Pos/neg cookies	Pos/neg garlic dressing
20000	10	10/0	10/0
200	10	10/0	10/0
20 ^a	10	10/0	10/0
2	10	0/10	0/10
0.2	10	0/10	0/10

^a Limit of detection

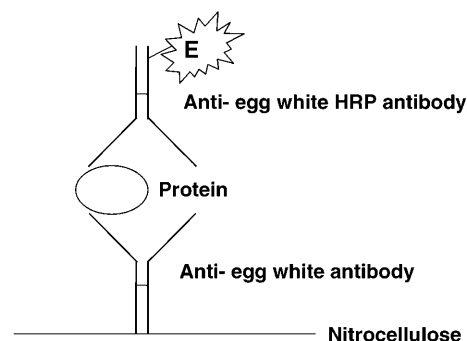


Fig. 2 The sandwich format was the optimal format for the dipstick ELISA. The anti-egg white-antibody was coated on the nitrocellulose membrane. The anti-egg white-horseradish peroxidase (HRP) antibody was used as detection antibody. E Enzyme (peroxidase)

sensitive than the egg white antibody. The optimal antibody combination for the sandwich ELISA was the egg white antibody as coating antibody and the egg white-HRP antibody as detection antibody (Fig. 2).

No cross-reactions could be observed between the egg proteins and the antibodies used or vice versa; nor were any matrix effects observed. In nearly all analyzed foods where egg proteins had been declared, they were detected by the dipstick.

Currently, it is not possible to perform a quantitative analysis. The limit of detection was measured in two different matrices (butter biscuits and garlic dressing). The matrices were spiked with five concentrations of egg standard from 0.2 µg/kg to 20 mg/kg. Table 2 shows that the limit of detection is about 20 µg/kg for the above-mentioned antibody combination. At a concentration below 2 µg/kg no egg protein was recovered.

Table 3 shows the list of analyzed food samples including ingredients; the samples where egg protein could be detected are marked with +.

Problems appeared when detecting egg yolk as a colorant in pudding and egg as a binder in fried minced

Table 1 Cross-reactions between the three different antibodies. X Cross-reactions, – no reactions, AB antibody, HRP horseradish peroxidase

	Whole egg AB	Egg white AB	Ovalbumin AB
Whole egg AB HRP	X	X	X
Egg white AB HRP	X	–	–
Ovalbumin AB HRP	X	–	–

Table 3 List of samples with ingredients and detectable egg protein

	Samples	Ingredients	Egg found+/-
Pasta	Spaghetti 1	Durum wheat, 3 eggs/kg noodles	+
	Spaghetti 2	Durum wheat, water	-
	Soup noodles	Durum wheat, 2.25 egg yolks/kg noodles	+
	Spätzle	Durum wheat, salt, 4 eggs/kg noodles	+
	Whole wheat spaghetti	Whole durum wheat, water	-
Biscuits	Chocolate 1	Wheat flour, fat, sugar, chocolate, liquid whole egg, glucose syrup, milk powder, candied fruits	+
	Chocolate 2	40% chocolate, wheat flour, fat, sugar, skimmed milk powder, butter, whole egg powder	+
	Nut 1	Wheat flour, fat, sugar, hazelnuts, liquid whole egg, glucose syrup, milk powder, candied fruits	+
	Butter biscuits 1	Wheat flour, butter (17%), sugar, glucose syrup, milk powder, whole egg powder, salt	+
	Butter biscuits 2	Wheat flour, butter (12%), sugar, whey product, milk powder, emulsifier	-
	Flaky pastry 1	Wheat flour, fat, sugar, salt, milk powder, skimmed milk powder, milk, sugar	-
Cakes and pastries	Cake 1	Whole egg (27%), sugar, wheat flour, fat, glycerin, sorbitol, sweet whey powder, salt, lecithin	+
	Cake 2	Wheat flour, sugar, whole egg, oil, whey, cacao, emulsifier, baking powder, salt	+
	Chocolate cake	Oil, sugar, wheat flour, maize starch, cacao powder, fructose, salt, emulsifier, sorbic acid	+
	Croissants 1	Wheat flour, egg, sugar, butter, milk, glucose syrup, yeast, salt, emulsifier	+
	Croissants 2	Wheat flour, margarine, whey, sugar, yeast, emulsifier, lecithin	-
Pudding	Pudding 1	Milk, water, sugar, cream, modified starch, carrageen, gelatine, colour: egg yolk	-
	Pudding 2	Milk, whey, cream, sugar, modified starch, glucose syrup, carrageen, gelatine, colour: beta-carotin	-
Dressings	Mayonnaise 80%	Sunflower oil, water, egg yolk (6.2%), salt, sugar, vinegar, mustard, lemon juice, lactic acid	+
	Garlic dressing 1	Water, oil, garlic, vinegar, sugar, milk protein, salt, mustard, egg yolk, modified starch, thickener, spices	+
	Garlic dressing 2	Water, sunflower oil, vinegar, sugar, garlic (5%), mustard, egg yolk, salt, spices, starch, xanthan	+
	Garlic dressing 3	Water, oil, sugar, vinegar, skimmed milk yoghurt, garlic (4%), whey powder, salt, spices, xanthan, starch, citric acid, skimmed milk powder, modified starch, lutein	-
Breaded frozen foods	Breaded fish	Polar cod (65%), breadcrumbs, oil, wheat flour, starch, salt	-
	Breaded chicken	Chicken breast (50%), water, breadcrumbs, oil, wheat flour, salt, lemon juice, yeast extract, onion powder	-
Meat products	Minced meat	Pork and beef, onion, breadcrumbs, egg, salt, maltodextrine, dextrose, spices, oil	-
	Liver dumpling	Liver of beef (33.4%), water, roll, bacon, spleen, onion, bread crumbs, egg, salt, spices	+
	Raw minced meat	Pork, beef	-

meat. In both cases the cause might be the low concentration of egg proteins, especially in pudding where only egg yolk had been used. After spiking the food samples with egg protein standard, the possibility that matrix effects were responsible for these false negative results could be excluded.

For the commercial application of the dipstick, the stability of the antibodies on nitrocellulose must be ensured. Ready-for-use dipsticks stored for 3 months at 6 °C showed complete function.

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