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Specific IgE as the best predictor of the outcome of challenges to baked milk and baked egg

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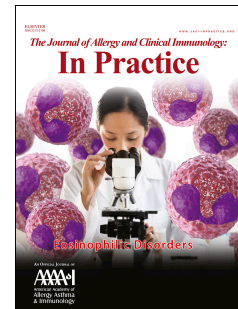
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Clinical Communication

Title: Specific IgE as the best predictor of the outcome of challenges to baked milk and baked egg

Short title: Predicting tolerance to baked milk and egg

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Clinical Implications (40 words): Specific IgE performed better than SPT to predict reactivity to milk and egg during challenges with 50% positive predictive value cut-offs being useful to support the decision of whether to challenge to baked milk and egg to assess for tolerance.

To the Editor

Cow's milk and egg allergies are common in childhood, affecting about 1.9-3%¹ and 0.5–2.5%² of young children respectively, but are often outgrown^{3, 4}. Oral food challenge (OFC) is the gold standard to diagnose milk and egg allergies and to assess resolution; however, OFCs are resource-intensive and involve the risk of inducing an allergic reaction of unpredictable severity. There has been a paradigm shift in the management of milk and egg allergies in view of recent studies showing that about 77-83% of milk-allergic patients tolerate baked milk (BM) and 75% of egg-allergic patients tolerate baked egg (BE)^{5, 6}, with many centers offering OFCs to assess tolerance to BM and BE as a means of encouraging inclusion of these foods to help broaden the diet, improve quality of life, and possibly assist tolerance acquisition^{6, 7}. In this study, we aimed to define predictors of clinical reactivity during OFCs to milk or egg, both baked and non-baked, to guide clinical decision making about when to refer for OFC to confirm tolerance.

Clinical records of patients who underwent OFC to cow's milk (baked or fresh) or egg (baked or cooked) between January 2014 and December 2016 were reviewed to assess OFC outcomes compared to SPT (Stallergenes, France/ ALK-Abelló, Denmark) and sIgE (ImmunoCAP, Thermofisher, Uppsala) tested prior to OFC. Referrals for OFCs were made at clinician's discretion, depending on the clinical history and allergy test results. Absence of reaction in the past year, SPT<5mm and sIgE<2 KU/L or discrepancy between history and allergy test results were the main indications for OFC. OFCs consisted of 4 doses of the challenge food administered openly after a baseline set of observations and physical examination that were repeated about 20 minutes after each dose up to a cumulative amount of 3.6g of milk protein for BM, 8.33g of milk protein for fresh milk (FM), 3.7g of egg protein for BE and 5.56g of egg protein for cooked egg OFCs, as long as no reaction developed. If patients' developed signs of an allergic reaction, the OFC was stopped, medication given and the patients were advised to avoid the food in the diet. See Online Repository for characteristics of the study population (Table E1) and details about the OFC procedure (Table E2 and Figures E1-2) and statistical analyses.

Over the 3-year period, 462 children underwent OFC to milk or egg and 94 (20%) had a positive OFC (Figure 1). Three (2%) of milk and 21 (7%) of egg challenges were inconclusive, due to refusal to eat the entire portion of challenge food and were excluded from the analysis. Overall, the OFCs were well tolerated, with only 6 (1%) requiring intramuscular adrenaline (Table E3): 2 to BM, 1 to FM and 3 to BE. There was no significant difference in the presence of intermittent or persistent asthma (defined as per GINA guidelines) between children who reacted and those who tolerated the food (FM: $p=0.481$, BM: $p=0.921$, cooked egg (CE): $p=0.476$, BE: $p=0.628$) or

between children who experienced anaphylaxis and those who experienced milder symptoms during OFC.

The OFC was used as the gold-standard to define allergy to each of the foods tested. We compared the results of SPT and specific IgE between subjects who had negative and positive OFC (Tables E4-5). Children who reacted to BM had higher SPT to cow's milk extract and higher milk-sIgE. Children who reacted to FM also had higher milk-sIgE. In children who reacted to BE, SPT to egg extract, SPT to raw egg and sIgE to egg white were higher compared to those who did not react. Only SPT to raw egg and the difference in SPT to egg extract and raw egg were significantly different between CE-allergic and CE-tolerant patients. Data for sIgE to milk and to egg individual allergen components was limited as these are not routinely used in our current clinical practice. ROC curve analyses were performed to assess the utility of each test to predict the outcome of OFC and cut-off points with 100% and 50% PPV were determined where possible (Figure E3, Tables 1 and E6).

This was a large study of well-characterized patients, all submitted to OFC, which allowed to assess the utility of SPT and sIgE to predict the outcome of OFCs and to identify cut-off levels with approximately 50% PPV that can support the decision of when to challenge to BM and BE, in our patient population. This is the first study looking at the clinical utility of SPT and sIgE in the context of BM and BE in our center, which is a WAO-accredited Allergy center and one of the largest food allergy centers in the world, and one of the very few studies looking at baked milk and egg OFCs performed in the United Kingdom or Europe. The larger proportion of patients being challenged to BM/BE reflects our recent change in practice of proactively assessing tolerance to the baked forms of these foods as opposed to strict avoidance of all forms of milk and egg advised in the past. In addition, once a BM/BE challenge has been undertaken, it is not common practice in our unit to refer straight on for a FM/CE OFC as the diet has already been expanded and the quality of life improved. Due to the higher number of patients challenged, the cut-offs generated for BM/BE are therefore more robust than for FM/CE. The low rate (18% milk, 22% egg) of positive OFC may reflect our proficiency in predicting allergic reactivity or alternatively may reflect a more conservative approach to challenge referrals. Our overall anaphylaxis rate was lower than in other series, which is likely to reflect differing criteria for OFC referral and/or practice for administration of adrenaline.

The dosing schedule for BM and BE challenges was based on age-appropriate portions of these foods to ensure a robust definition of patient phenotype and patients' safety when eating shop-bought and home-baked products that can contain variable quantity of the baked allergen. The fact

that the proportion of positive OFCs was low despite the higher protein content of the challenge doses further suggests that our population was low risk compared with other published series^{5, 6, 8, 9}. Consistent with previous studies, gender, age, atopic co-morbidities including the presence of asthma were not able to predict clinical reactivity during OFC^{6, 8, 9}. We found that SPT wheal size and sIgE levels were generally higher for allergic children. Surprisingly, SPT did not perform as well as we expected and this could be in part because in our clinic, SPT is at the core of the decision of whether to refer for OFC. Many studies have looked at the ability of allergy tests to predict positive challenges (e.g. 95% PPV), which is valuable to confirm allergy, but fewer studies have looked at cut-offs to determine whether to challenge to assess resolution. We identified 50% PPV cut-offs for sIgE milk and egg white in relation to tolerance to BM and BE that can support timely decisions on referral for OFC in the future. Further studies are needed to validate our cut-offs, ideally in a larger prospective study to help establish even more reliable predictors of challenge outcomes.

The study focused on a selected population of children referred for an OFC within our service and does not include all children being assessed for milk and egg allergies; therefore, the cut-off points may have limited generalizability. Compared with other studies^{5, 6}, our cut-offs tended to have lower NPV and higher PPV, suggesting that our population were more likely to react at a given sIgE level compared to other populations. In order to include a population of children that is more representative of all children with suspected food allergy seen in specialized clinics, we would have had to include highly sensitized patients. However, this would have meant that we had to either challenge children at high-risk of reaction, which has ethical limitations, or determine the allergic status of children based solely on SPT and specific IgE results and not on the gold-standard OFC, which has its own limitations. This is why our data is important, as it reflects the decision making process that takes place in a real life clinic scenario. Only an unbiased approach in a purpose-designed diagnostic study in which patients undergo all tests including OFC would allow for a precise determination of global diagnostic cut-off points for the various tests. The ongoing BAT2 Study (NCT03309488) will define more generalizable cut-offs for the diagnosis of BM and BE that allow dispensing OFCs with a higher degree of certainty.

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Table:

Table 1. Optimal cut-offs for skin prick test to extract and specific IgE to cow's milk or egg white and 100% PPV cut-offs (to confirm allergy) and 50% PPV cut-offs (to determine whether to challenge) for specific IgE to cow's milk or egg white. Optimal cut-offs were defined as the best balance between sensitivity and specificity and calculated based on the largest Youden index

		Baked milk allergy	Fresh milk allergy	Baked egg allergy	Cooked egg allergy
<i>SPT to extract</i>	AUC ROC 95% (CI)	0.66 (0.54-0.78)	0.56 (0.37-0.76)	0.6 (0.51-0.68)	0.62 (0.41-0.82)
	Optimal cut-offs	2mm S =76% Sp= 55% PPV=27% NPV= 91%	2mm S=29% Sp= 84% PPV=33% NPV=81%	4mm S=57% Sp=62% PPV=39% NPV= 77%	3mm S=43% Sp=81% PPV=20% NPV= 93%
<i>Specific IgE</i>	AUC ROC 95% (CI)	0.72 (0.60; 0.84)	0.72 (0.59; 0.85)	0.74 (0.64; 0.83)	0.75 (0.42; 1.00)
	Optimal cut-offs	3.06 KU/L S=75% Sp=69% PPV=92% NPV= 35%	0.3 KU/L S=89% Sp=54% PPV=28% NPV=96%	2.81 KU/L S=67% Sp=81% PPV=84% NPV=61%	0.94 KU/L S=67% Sp=83% PPV=20% NPV= 98%
	100% PPV cut-offs	84.9 KU/L (100% PPV)	6.60 KU/L (100%PPV)	ND	ND
	50% PPV cut-offs	9.34 KU/L	3.31 KU/L	1.61 KU/L	ND

Abbreviations: AUC ROC, area under the receiver operator characteristic curve; CI, confidence interval; SPT, skin prick test; S, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

Figure and Figure Legend:

Figure 1. Oral food challenges performed within the 3-year period of the study. For qualitative variables number and percentages and for quantitative variables median and interquartile range are indicated. SPT are represented in mm and specific IgE in KU/L.

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462 Oral Provocation tests conducted between Jan. 2014 - Dec. 2016

181 milk challenges

- 111 baked milk
- 67 fresh milk
- 3 inconclusive

281 egg challenges

- 178 baked egg
- 83 cooked egg
- 20 inconclusive

33 (18%) positive challenges

- 20 (18%) baked milk
- 13 (19%) fresh milk

61 (22%) positive challenges

- 54 (30%) baked egg
- 7 (8%) cooked egg

Baked milk positive challenges

SPT milk extract 3(1;5)
SPT fresh milk 5(4;1)
Specific IgE milk 5.91
(4.47;10.38)

Fresh milk positive challenges

SPT milk extract 0 (0;3)
SPT fresh milk 2 (0;4)
Specific IgE milk 0.75
(0.93;1.68)

Baked egg positive challenges

SPT egg white extract 5(2;8)
SPT raw egg 11 (7;15)
Specific IgE egg white
3.79 (2.76;4.83)

Cooked egg positive challenges

SPT egg white extract 5.5 (3;8)
SPT raw egg 2 (0;5)
Specific IgE egg white
1.0 (0.33;1.77)

Ingredients

Makes 8 fairy cakes (60g)

125g (4 oz) self raising flour.

125g (4 oz) soft butter or margarine.

125g (4 oz) caster sugar.

Two medium sized eggs whisked.

30g skimmed milk powder e.g. Marvel.

Method

- Preheat the oven to 180C/350F/Gas 4.
- Cream the butter/margarine and the sugar together until light and fluffy.
- Add the whisked egg to the mixture and slowly fold in the self-raising flour and skimmed milk powder.
- Spoon the mixture into eight paper cases sitting in a bun tin.
- Cook for approximately 15 - 20 minutes until risen and bouncy to touch or until a skewer comes out clean when inserted.

Ingredients

Makes 8 fairy cakes (60g)

1.

125g (4 oz) self-raising flour

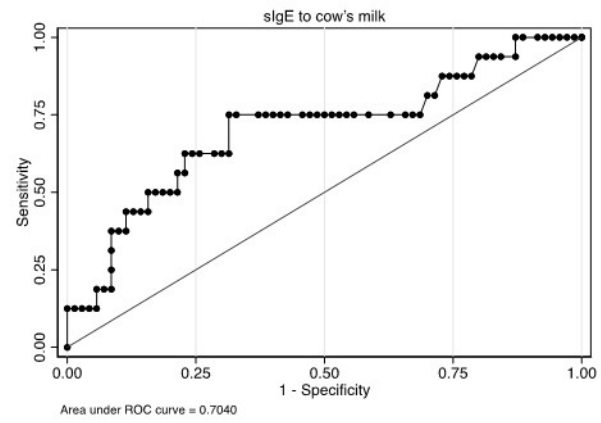
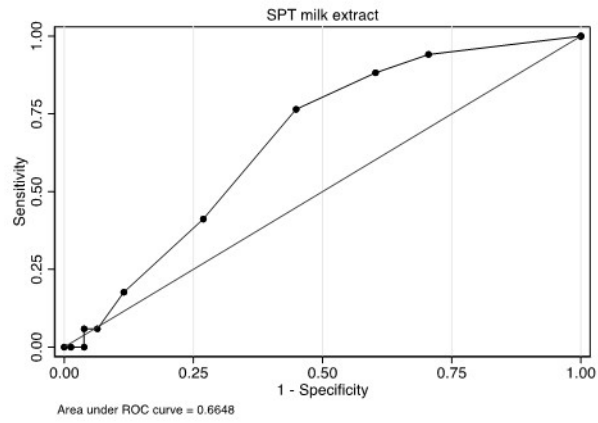
125g (4 oz) soft butter or margarine (use milk/soya free if required)

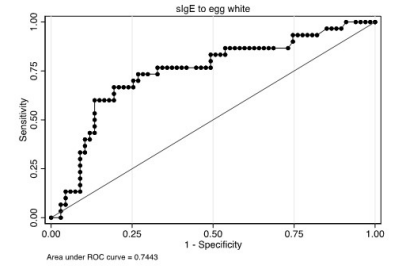
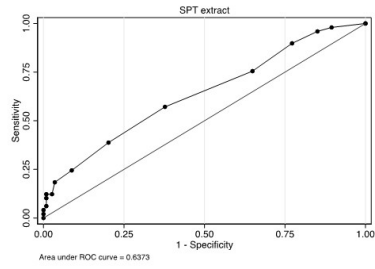
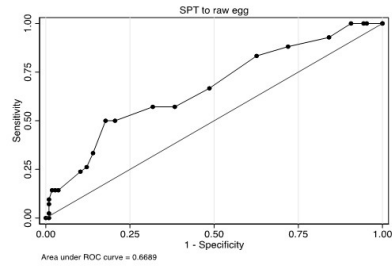
125g (4 oz) caster sugar

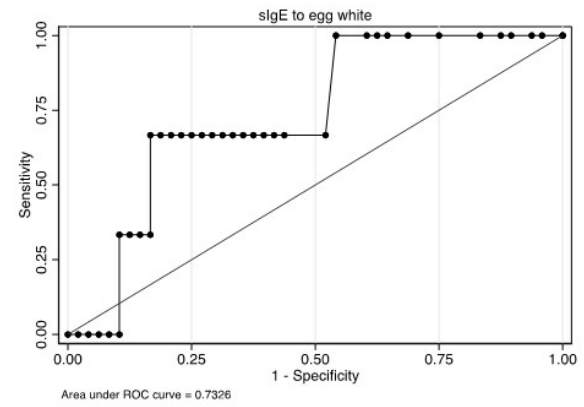
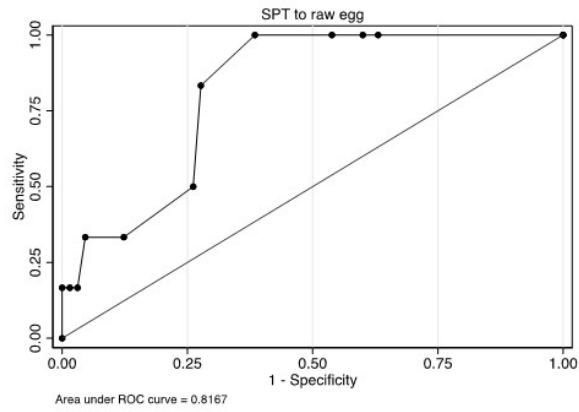
Two medium eggs whisked

Method

- Preheat the oven to 180C/350F/Gas 4.
- Cream the butter/margarine and the sugar together until light and fluffy.
- Add the whisked egg to the mixture and slowly fold in the self-raising flour.
- If the mixture becomes too thick, add a small amount of milk or your child's usual milk substitute (e.g. soya/oat/coconut milk).
- Divide the mixture equally between **eight** paper cases sitting in a bun tin.
- Cook for approximately 15 minutes in the top of the oven until risen and bouncy to touch or until a skewer comes out clean when inserted.







Online Repository:

E-Methods:

Skin prick testing and specific IgE testing

Skin prick test (SPT) was performed as previously described[1], using metal lancets and commercial allergen extracts (Stallergenes, France/ALK-Abelló, Denmark) as well as fresh milk (FM) and raw egg prepared on the day of the SPT. Whole milk was used for SPT to FM. In the SPT to raw egg, the preparation included both egg white and egg yolk. The difference in millimeters between the weal diameters to FM and milk extract and between raw egg and egg extract were calculated. Specific IgE to milk or egg white was tested using ImmunoCAP (ThermoFisher, Uppsala). The oral food challenge (OFC) referrals were done at the clinician's discretion, depending on the clinical history and allergy test results.

Oral food challenges

Patients underwent open OFC using standardized up-dosing schedules (Table E2). Fresh full fat cow's milk and scrambled egg or omelette cooked on the cooker for 5 minutes were used for FM and cooked egg OFC, respectively. Standardized recipes for milk and/or egg containing cupcakes were used for baked milk and baked egg OFCs (Figures E1-2). OFCs were considered negative when the child successfully consumed the total cumulative dose without displaying any signs of an allergic reaction and were considered positive if objective signs of an allergic reaction developed. If so, the OFC was stopped, and patients were treated according to local guidelines and at the discretion of the clinician. Tolerant children were recommended to incorporate the tolerated food form into their diet at least twice weekly and allergic children were recommended to avoid the culprit food.

Statistical analyses

For determining the significance of differences between-groups the Wilcoxon rank sum test was used. The performance of allergy tests was examined against the allergic status using receiver-operating characteristic (ROC) curve analyses, with their respective sensitivity, specificity and predictive positive and negative values. Optimal cut-off points were based on the largest Youden Index and the 100% and 50% positive predictive value (PPV) cut-offs

1 were determined by approximation. Statistical analyses were done using Stata statistical
2 software; release 15 (StataCorp, College Station, TX, 2017) and p values of less than 0.05
3 were considered statistically significant.

4

5

Table E1. Baseline clinical characteristics of the study population assessed for milk or egg allergies.

Clinical features	Milk (n=181)	Egg (n=281)
Median age (years)	5.3	6.16
Other food allergy	172 (97%)	210 (80%)
Atopic eczema	134 (75%)	159 (61%)
Allergic rhinitis	53 (30%)	57 (22%)
Persistent asthma	36 (20%)	26 (10%)
Intermittent asthma	20 (11.2%)	51 (19%)

Table E2. Scheduled doses of milk and egg challenge foods.

	Doses of challenge food	Dose of food protein (g)
Baked milk*	Dose 1: ¼ fairy cake Dose 2: ½ fairy cake Dose 3: ¾ fairy cake Dose 4: 1 fairy cake Total cumulative dose: 2 ½ fairy cakes	0.33g milk protein 0.65g milk protein 0.98g milk protein 1.3g milk protein 3.26g milk protein
Fresh milk	Dose 1: 5ml Dose 2: 10ml Dose 3: 30ml Dose 4: 200ml Total cumulative dose: 245ml	0.17g milk protein 0.34g milk protein 1.02g milk protein 6.8g milk protein 8.33g milk protein
Baked egg*	Dose 1: 1/4 fairy cake Dose 2: 1/2 fairy cake Dose 3: 3/4 fairy cake Dose 4: 1 fairy cakes Total cumulative dose: 2 1/2 fairy cakes	0.47g egg protein 0.94g egg protein 1.41g egg protein 1.875g egg protein 3.7g egg protein
Cooked egg	Dose 1: 0.5g Dose 2: 4g Dose 3: 10g Dose 4: 30g Total cumulative dose: 44.5g	0.06g egg protein 0.5g egg protein 1.25g egg protein 3.75g egg protein 5.56g egg protein

*for recipes used for baked milk and baked egg challenge foods see figure E2.

Table E3. Characteristics of allergic reactions during oral food challenges (OFC) to milk or egg

Symptoms	OFC to milk	OFC to egg
Nausea & abdominal pain	6 (18%)	28 (46%)
Pruritus	12 (36%)	27 (44%)
Rash	20 (26%)	16 (26%)
Vomiting	2 (6%)	22 (36%)
Urticaria	4 (12%)	17 (28%)
Rhinitis	7 (21%)	15 (25%)
Possible neurological symptoms*	5 (15%)	7 (11%)
Stridor	5 (15%)	6 (10%)
Wheeze	6 (18%)	1 (2%)
Treatment administered	OFC to milk	OFC to egg
Anti-histamine	33 (100%)	61 (100%)
Salbutamol	8 (24%)	2 (3%)
Intramuscular adrenaline	3 (9%)	3 (5%)
Prednisolone	1 (3%)	2 (3%)

*Examples of neurological symptoms are dizziness, feeling queasy, change in behavior.

Table E4. Allergy test results comparing children with positive and negative challenges to baked and fresh cow's milk. Median, interquartile range and p-values using Wilcoxon rank sum test are represented for SPT and specific IgE results. P values <0.05 are marked in bold.

Baked milk challenges (n=111)				Fresh milk challenges (n= 67)		
	Positive, (n=20)	Negative (n=91)	p value	Positive (n = 13)	Negative (n=54)	p value
Age (years)	4.4 (2.3; 8.6)	4.5 (2.3; 7.1)	0.860	3.7 (2.6; 6.2)	4.3 (2.7; 7.3)	0.788
History of reactions to milk	35% (7)	40% (36)	0.705	15% (2)	19% (10)	0.791
SPT fresh milk (mm)	5 (4; 1)	7 (5.5; 8.5)	0.473	2 (0; 4)	2 (1; 3)	0.907
SPT milk extract (mm)	3 (1; 5)	2 (0; 4)	0.031	0 (0;3)	0 (0; 1)	0.850
SPT differenc e fresh milk- milk extract (mm)	3 (3; 3)	5 (5; 5)	0.076	0 (0; 2)	1 (0; 2)	0.282
Specific IgE to milk (KU/L)	5.91 (4.47; 10.38)	1.68 (2.10; 3.77)	0.011	0.75 (0.93; 1.68)	0.27 (0.22; 0.49)	0.011

Table E5. Allergy test results comparing children with positive and negative challenges to baked and cooked egg. Median and interquartile range are represented for SPT and specific IgE results. P values <0.05 are marked in bold.

	Baked egg challenges (n=179)			Cooked egg challenges (n=83)		
	Positive (n=54)	Negative (n=125)	p value	Positive (n= 7)	Negative (n= 76)	p value
Age (years)	6.0 (3.2; 8.2)	4.3 (2.5; 8.2)	0.157	11.6 (4.9; 13.3)	4.1 (2.1; 8.4)	0.046
History of reactions to egg	11.1% (6)	13.6% (17)	0.974	28.57% (2)	28% (21)	0.974
SPT raw egg (mm)	11 (7; 15)	7 (5; 9)	0.001	5.5(3;8)	3 (0; 6)	0.009
SPT egg extract (mm)	5 (2; 8)	4 (2; 6)	0.005	2 (0; 5)	1 (0; 3)	0.283
SPT differenc e raw egg-egg extract (mm)	4 (2; 6)	3 (2; 5)	0.862	3.5 (3; 4)	0 (0; 2)	0.010
Specific IgE to egg white (KU/L)	3.79 (2.76; 4.83)	1 (0; 2.12)	<0.001	1 (0.33; 1.77)	0.31 (0; 0.68)	0.180

Table E6. Optimal cut-offs for skin prick test to fresh milk/raw egg and difference between SPT to fresh and SPT to extract.

		Baked milk allergy	Fresh milk allergy	Baked egg allergy	Cooked egg allergy
SPT fresh milk/raw egg	AUC ROC 95% (CI)	0.56 (0.53; 0.6)	0.54 (0.41; 0.67)	0.66 (0.5; 0.75)	0.81 (0.75; 0.87)
	Optimal cut-offs	1 mm S=100% Sp = 12% PPV=19% NPV=100%	4mm S= 23% Sp=85% PPV=27% NPV= 82%	11mm S=50% Sp=82% PPV=53% NPV= 81%	3mm S=100% Sp = 62% PPV=19% NPV= 100%
Difference between SPT to fresh and SPT to extract	AUC ROC 95% (CI)	0.55 (0.46-0.64)	0.5	0.58 (0.49-0.66)	0.77 (0.70-0.84)
	Optimal cut-offs	10mm S=13% Sp=96% PPV=40% NPV= 85%	12mm S=0% Sp=100% PPV=0% NPV=50%	2mm S=76% Sp=40% PPV=34% NPV= 80%	0mm S=100% Sp=54% PPV=20% NPV= 100%

Abbreviations: AUC ROC, area under the receiver operator characteristic curve; CI, confidence interval; SPT, skin prick test; S, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

E-Figure Legends:

Figure E1. Recipe for standard homemade cupcake for baked milk challenge

Figure E2. Recipe for standard homemade cupcakes for baked egg ch

Figure E3. Receiver-operator characteristic (ROC) curves for the different tests regarding

(A) baked milk, (B) fresh milk, (C) baked egg and (D) cooked egg challenge outcomes.

SPT, skin prick test; sIgE, specific IgE

E-References:

- E1. Santos, A.F., et al., *Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children*. J Allergy Clin Immunol, 2014. **134**(3): p. 645-52.