

A Novel Double-Blind, Placebo-Controlled Food Challenge Matrix for Milk and Raw Egg

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Keywords

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Abstract

Background: The double-blind, placebo-controlled food challenge (DBPCFC) is still considered to be the gold standard in food allergy diagnosis. This test is however not common practice in routine due to several practical limitations, especially for non-IgE-mediated food allergy with its typical delayed food allergic reactions. **Objective:** The aim of this study was to develop and evaluate DBPCFC matrices for the diagnosis of milk and egg allergies which can be applied at home for the diagnosis of delayed food allergic reactions. The main focus was the blinding of milk and raw egg and the development of matrices which can be prepared and consumed conveniently at home with a sufficiently long shelf life (+/- 6 months or longer). **Methods:** A sensory test evaluated the blinding of the egg and milk in the matrices. The microbiological analysis confirmed the safety and stability of

the developed matrices. To assess the applicability of the matrices, a pilot DBPCFC study for milk including 7 patients was conducted. **Results:** Sensory tests confirmed that the masking of the allergenic ingredients was sufficient. Microbial safety and stability of the matrices were confirmed up to 6 months of storage at ambient temperatures in the dark. The DBPCFC for milk showed different outcomes and proved its applicability for use at home. **Conclusion:** A novel stable DBPCFC matrix for milk and raw egg has been developed that allows convenient use at the patients' home.

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Introduction

Egg and milk allergy are among the most common food allergies occurring in infants and young children, with global estimated prevalences of 0.5–2.5% and 2–3%, respectively [1, 2]. Egg and milk allergy are usually outgrown during childhood, but may persist during adolescence or

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adulthood [1, 3]. Both allergies are defined as immunologically mediated adverse reactions induced by specific allergenic proteins. Reactions of both allergies might be either IgE-mediated, non-IgE-mediated, or mixed, whereby the IgE-mediated allergy is the most common [1, 2].

For IgE-mediated allergies, serum-specific IgE and skin prick testing are frequently used for diagnosis. However, both methods cannot predict the severity of an allergic reaction, they merely indicate sensitization [2]. For the non-IgE-mediated food allergy, no validated diagnostic methods apart from exclusion-provocation tests are available. This type of food allergy encompasses a wide range of signs and symptoms, such as atopic dermatitis, gastrointestinal disorders, celiac disease, and lung disorders [4]. Thus, with its diverse, subjective, and nonspecific manifestations of symptoms, the diagnosis is not straightforward [4]. Nevertheless, a correct and reliable allergy diagnosis is of utmost importance because prevention of allergic reactions relies on the avoidance of the allergenic ingredient. This elimination diet has a significant impact on quality of life [5]. Furthermore, it has been noticed that parentally perceived adverse reactions might lead to allergen-free diets without an appropriate diagnosis. The potential overestimation of the role of the allergenic ingredients (e.g., milk) as the cause of particular symptoms experienced by their children can lead to unnecessary, yet often radical diet restrictions given the omnipresence of egg and milk in food products [6]. As reported in a systematic review in 2014 [7], the self-reported prevalences of cow's milk and egg allergies were 6 and 2.5%, whereas the prevalences of food challenge-defined allergies were 0.6 and 0.2%, respectively.

Since non-IgE-mediated allergic symptoms are most often characterized by delayed allergic reactions, which occur between 4 and 48 h after consumption, only an elimination diet with an at home reintroduction (e.g., by an oral food challenge) can confirm its possible presence. The oral food challenge provides also more insight concerning the severity and resolution of the particular allergy. Furthermore, a negative test outcome, followed by an open exposure of the particular allergenic ingredient, helps to reduce the anxiety concerning this allergenic ingredient and confirms that avoidance is not needed. This leads to an improved variation in the patients' diet and as such enhances their quality of life [5].

Of the oral food challenges, the double-blind, placebo-controlled food challenge (DBPCFC) is considered to be the gold standard in food allergy diagnosis. The blinding of the allergenic ingredients will minimize the potential psychological placebo effects for the patients and the

healthcare professionals [5]. However, due to practical limitations (e.g., time-consuming and burdensome procedure), the DBPCFCs are not performed routinely and more often open food challenges are performed. Most DBPCFC studies to diagnose IgE-mediated food allergies are conducted in clinical settings, where emergency care is available promptly and effectively when a severe allergic reaction occurs. Nevertheless, only a few DBPCFC studies to diagnose late-onset reactions due to non-IgE-mediated reactions have been conducted at the patients' home for a longer period [8]. Also, no standardized protocol exists despite numerous efforts [8, 9]. Yet, this standardization is needed to facilitate its use and to allow comparison of results obtained from different clinical centers [8].

Although several recipes have been developed in earlier studies for milk and egg [10–12], validated matrices are needed which are easy to prepare at home and available for consumption during longer periods (+/– 2 weeks or more) to monitor late-onset reactions. For milk, some studies report the use of hydrolyzed infant formulas [10, 11], which are convenient to prepare in a standardized way at home. However, these are often disliked by older children and especially by adults, who are not used to these formulas. For egg, no validated matrices are reported yet containing the full egg (egg white and yolk) which are convenient to prepare at home. For instance, in the study of González-Mancebo et al. [12], only egg white was used as the allergenic ingredient.

In this study, the focus was laid on the development and evaluation of DBPCFC matrices for the diagnosis of milk and egg allergies, which can be prepared conveniently at home without the patients being able to unblind these preparations (and if children perform this test, neither by the parents who prepare the matrices). Furthermore, they must be appreciated by both adults and children. Several criteria such as blinding of the allergenic ingredients, taste, and microbial stability were also taken into account. Finally, attention was paid to the fact that no other major allergens, which are listed in EU Regulation No. 1169/2011, were present in the matrices as ingredients. As proof of concept, milk DBPCFCs were performed at home by selected adults and children.

Subjects and Methods

Preparation of the DBPCFC Powder Mix

Shelled raw eggs (Hulstaert & Cie Nv., Sint-Niklaas, Belgium) and semi-skimmed, lactose-free milk (Dilea Zero Lactose, Lillois-Witterzée, Belgium) were freeze-dried (Epsilon 2-10 D LSC; Martin Christ, Osterode am Harz, Germany), vacuum packed, and

Table 1. Ingredients used for the DBPCFC matrices for milk and egg

Product	Brand	Milk		Egg	
		placebo, g/portion	verum, g/portion	placebo, g/portion	verum, g/portion
Vanilla sugar ¹	Imperial	8.00	8.00	8.00	8.00
Brown sugar ¹	Candico	3.00	2.40	3.00	2.50
Stevia ¹	Pure Via	6.50	5.82	4.90	5.2
Cacao 100% ¹	Callebaut	12.00	13.00	11.00	11.00
Cold thickener (Quelli) ²	Dawn	1.40	1.10	1.75	1.40
Sunflower Lecithin Sun 400 organic ³	Lecico	0.30	0.30	0.30	0.30
Vanilla aroma ³	Dawn	0.46	0.34	0.50	0.38
Caramel syrup ¹	Funcakes	1.34	1.30	1.10	1.20
Rice Flakes Vanille ¹	Olvarit	2.20	2.50	2.50	3.00
Nutramigen AA ⁴	Mead Johnson	3.40	5.50	0.00	0.00
Milk	Dilea Zero Lactose	0.00	5.00 ^a	–	–
Egg	Hulstaert & Cie Nv.	–	–	0.00	3.41 ^b
Water	–	60.00	60.00	65.00	65.00
Total volume per full portion	98.6	105.3	98.1	101.4	98.6
Total volume per half portion	49.3	52.6	49.0	50.7	49.3

DBPCFC, double-blind, placebo-controlled food challenge. ¹ No allergens were mentioned on the product information sheet. ² Has precautionary labeling on the product information for most of the major allergens. ³ Explicitly mentioned that it contains no allergens. ⁴ Is an amino acid-based formula for babies with severe cow's milk protein allergy or with multiple food allergies; it is an extensively hydrolyzed formula by completely breaking down the present proteins in amino acids. ^a 5.00 g milk powder = 1.85 g proteins/portion = ±80 mL milk. ^b 3.41 egg powder = 1.59 g proteins/portion = 1/4 whole egg.

stored in the dark. The protein content was determined using Kjeldahl analysis (according to ISO 8968-2, conversion factor milk = 6.38 and egg = 6.25). The placebo and verum powder mixes were developed as presented in Table 1. The ingredients were purchased from local stores. The developed powders were kept in pots with screw caps at ambient temperatures in the dark (Gosselin, Deurne, Belgium).

Microbial Stability

Enumerations of *Escherichia coli* (AFNOR BRD-07/01-07/93 method), *Bacillus cereus* (ISO 7932), and detection of *Salmonella* (ISO 6579) were performed in the ISO 17025 accredited lab for food microbiology at the Belgian National Reference Lab at the Research Institute for Agriculture, Fisheries, and Food (ILVO), Melle, Belgium for all the developed powder mixes. Water activity (a_w) was tested using the Benchtop Water Activity Meter (Decagon Devices, Pullman, WA, USA) to predict microbial stability.

Sensory Testing

In phase I, a preliminary sensory test was conducted, for milk only, in sensory evaluation booths with 31 panelists who were already used to sensory tests in general. The main focus was the assessment of the taste and blinding of the milk, prior to further optimization of the matrices and larger-scale sensory tests for both milk and egg. Three desserts were given sequentially in different orders (at random combination of verum and placebo matrices). The panelist knew in advance that both the placebo and verum

matrices would be given at random. Between samples, water and crackers were given. After every tasting, they had to answer whether the dessert contained milk or not and whether this was a guess. If the allergenic ingredient was detected, they also had to answer whether this was by tasting and/or smelling. The data was collected using the FIZZ Biosystems software (Couternon, France).

In phase II, all the matrices (milk and egg) were evaluated with a consumer panel (90 panelists). The same protocol was performed as in phase I, with two samples: a placebo and verum sample were given separately, in different randomized orders. After every tasting, they had to answer the same questions as in phase I. Between the samples, water and crackers were given as well.

All the tests were analyzed using the χ^2 test with a 95% confidence interval. When there was statistically no difference between the answers obtained with the verum and placebo samples, the milk and egg were considered sufficiently blinded in the powder mixes. Panelists with food allergies were not allowed to participate.

DBPCFC Protocol

As proof of concept, 7 patients were carefully selected to perform the milk DBPCFCs (see Table 2 for the clinical parameters of the patients). These were patients with a diverse range of complaints (abdominal pains, eczema, etc.), whereby the cause is not known yet, who wanted to exclude or confirm a possible non-IgE-mediated milk allergy. All patients were devoid of an IgE-mediated milk allergy and anaphylactic signs or symptoms. The patients had to exclude the allergenic ingredients for a minimum of 2 weeks

Table 2. DBPCFC results of the different patients

Patient No.	Age, years	Sex	Symptoms/signs	Series	Duration of the test	Additional comments	Test outcome
1	2	M	abdominal pain and constipation in both series	1: verum 2: placebo	complete complete	more complaints during series 2	neg.
2	2.5	F	eczema, nausea, diarrhea in both series	1: verum 2: placebo	week 1 + day 1 of week 2 day 1–4 and 6–7 of week 1, week 2	since the test, milk has been introduced back into the diet without exacerbation	neg.
3	2.5	F	eczema of the chin and eyes and fatigue present in both series	1: placebo 2: verum	complete week 1 + day 1 week 2 (day 2 and 6 only 3 small spoons)		neg.
4	8	F	severe abdominal pain, diarrhea, vaginal secretion, headache, pale (day 1 → 14)	1: verum	complete		pos.
			no complaints (day 1 → 14)	2: placebo	complete		
5	39	F	itchy pubic area (day 1 → 14), urticarial and itchy neck region (day 6 → 14), headache (day 7), swollen eyes (day 9)	1: verum	week 1 + day 1 of week 2 (due to complaints)		
			small dots in the neck, itchy pubic area	2: placebo	complete	remaining diminishing complaints of series 1 due to too short pause between series (<1 week)	pos.
6	35	F	no complaints in both series	1: placebo 2: verum	complete complete		neg.
7	6	F	abdominal pain, nausea	1: verum 2: placebo	complete complete	no difference in complaints between both periods	neg.

DBPCFC, double-blind, placebo-controlled food challenge; neg., negative test outcome for milk; pos., positive test outcome for milk.

prior to the start of the test and had to keep this diet throughout the whole test period.

The patients received the placebo and verum DBPCFC series for milk at random order whereby each series contained 14 portions (7 half and 7 full portions), which was 1 portion per day for 2 weeks per series (Fig. 1). During the first week, the half portions had to be consumed and the second week the full portions (Table 1). However, the patients (or their parents) could stop the test whenever it started to be uncomfortable. Between each series, a pause was established depending on the severity of the symptoms/sign of the first series (+/– 1 week).

The portions had to be prepared at home, meaning that only a specific amount of water had to be added to the powder mixes (Table 1). Clear instructions were given how to prepare the matrices in an informative booklet provided together with the matrices. The portions had to be shaken vigorously and set at 4 °C for at least 1 h prior consumption. The freshly prepared portions had to be consumed the day itself.

All patients were advised by a trained and experienced dietitian on the content of a strict exclusion diet which was maintained throughout the trial. This advice consisted of a consultation, where a written information booklet of the diet was explained, and given to the parents/patients. No new food items were introduced during

Table 3. Sensory test results

Matrix		Phase I milk			Phase II milk			Phase II egg		
		tasting		total	tasting		total	tasting		total
		detected milk	did not detect milk		detected milk	did not detect milk		detected egg	did not detect egg	
Verum	count	11	20	31	57	33	90	42	48	90
	expected count ¹	12	19	31	54.5	35.5	90	38.5	51.5	90
Placebo	count	13	18	31	52	38	90	35	55	90
	expected count ¹	12	19	31	54.5	35.5	90	38.5	51.5	90
Total	count	24	38	62	109	71	180	77	103	180

¹ Total number (row). Total number (column)/total numbers = the expected counts are the projected frequencies in each cell if the null hypothesis is true (H0 = no statistically different frequencies in detecting the allergens in the verum and placebo series).

the trial. During the challenges, the patients (or parents) had to fill in an evaluation booklet to report observed signs and symptoms (type and number of times of stool, eczema, abdominal pain, vomiting, and any other additional complaints). Besides the symptoms, the participants also had to register each day how much of each portion they had consumed.

Results

Characterization of the DBPCFC Matrices

The final recipe of a cocoa powder mix is presented in Table 1. The freeze-dried milk (protein content: 37.01%) and egg (protein content: 46.7%) could be mixed homogeneously into the cacao powder without leaving visible particulates. The a_w values for the milk and egg placebo as well as for the milk and egg verum powder mixes were 0.474, 0.498, 0.465, and 0.450, respectively. Microbial counts of the same powder mixes were done just after preparation and 6 months after storage at ambient temperature in the dark. Also, the freeze-dried milk and egg were microbiologically analyzed separately. For all the tests, the results were <10.0 cfu/g for *E. coli*, 0/25 g for *Salmonella* sp., and <10.0 cfu/g for *B. cereus* even after 6 months of storage in the dark at ambient temperature.

Sensory Test

In the preliminary test results with milk (phase I), no significant difference was observed between the placebo and verum samples ($p = 0.397$; Table 3). Of the 31 panelists, 35 and 42% thought (whereof 46 and 39% guessed,

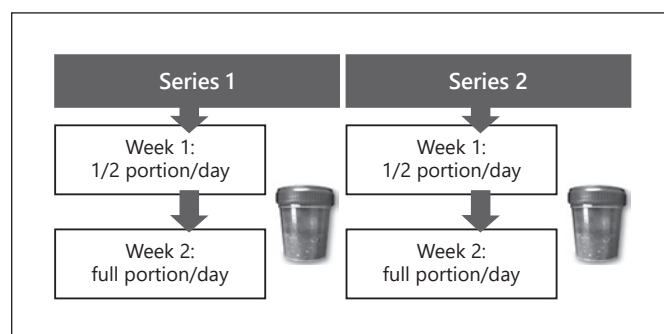


Fig. 1. Schematic overview of the DBPCFC protocol. Two different series (placebo or verum) are administered at two different periods. The code of the series is unknown to both the dietician/physician and the patients during the challenges. DBPCFC, double-blind, placebo-controlled food challenge.

respectively) that milk was present in the verum and placebo samples. In the group of panelists who detected milk in the verum and placebo samples, no significant differences were shown in smell and taste either ($p = 0.410$ and $p = 0.585$, respectively). Since most of the panelists appreciated the matrices and the milk was sufficiently blinded, a sensory test with a greater panel (90 panelists; phase II) was conducted for milk and egg. Since the matrices for egg were similar to those for milk and based on the results of phase I for milk, we decided to take the egg matrices directly to phase II of the sensory tests.

In phase II (Table 3), 63% (41% guessed) and 47% (47% guessed) thought that milk and egg were present in

the verum samples, respectively. For the placebo samples, 48 and 39% (whereof 43 and 40% guessed, respectively) thought that milk and egg were present. No significant difference existed between the placebo and verum samples ($p = 0.271$ and $p = 0.183$ for milk and egg, respectively). Of the panelists who detected milk and egg in the verum and placebo samples, no significant differences were seen between the verum and placebo samples either (smell and taste for milk and taste for egg: p values of 0.190, 0.320, and 0.248, respectively). Only for the smell of egg was a significant difference seen ($p = 0.042$).

DBPCFC Results

Two of the 7 patients (patients 4 and 5; Table 2) experienced both clear subjective and objective complaints during the verum challenges, which were absent during the placebo series. Patient 4 experienced abdominal pain, diarrhea, vaginal secretion, and headache during the verum series. Patient 5 had an itchy pubic area, urticarial and itchy neck region, headache, and swollen eyes. For the other patients, no distinct differences were seen between the verum and placebo series. Most of the patients found the portions tasteful to consume. For 2 children (patients 2 and 3), the portions were more difficult to consume and were not finished completely due to dislike.

Discussion

In this work, recipes for DBPCFC matrices suitable to blind milk and egg were developed whereby different criteria were taken into account.

The total milk and egg proteins consumed per full portion (1.85 and 1.59 g of proteins, respectively) were somewhat lower than those described in the work by Vlieg-Boerstra et al. [11] (2.8 and 4.0 g of proteins, respectively), where the focus was laid on the achievement of high top doses of allergens in DBPCFC matrices. For egg, this difference was higher, however, in this latter study; the eggs were baked at 150 °C for 30 min, which significantly reduced their allergenicity. Also, the recipe with egg was not suitable for DBPCFCs in young children because of the texture and total test volume. In our work, however, raw eggs were applied and the matrices will be applicable for younger children. For the protein portions of this study, a compromise had to be made between the total amount of the allergenic ingredient and the possibility to mask this ingredient. It is important to use recipes guaranteed for adequate blinding since unblinding can have an effect on the test outcome of the diagnosis.

A cold dessert was developed to minimize further processing steps which may alter the allergenicity of the food allergenic ingredients. The milk was lactose-free so that test results could not be confused with malabsorption lactose intolerance [13]. Further, both casein and whey proteins had to be present since both can elicit food allergic reactions [14]. UHT milk was chosen because unprocessed milk consumption is rare in developed countries, especially when used in food products [15]. The milk is hereby mostly heated, whereby UHT processing dominates in Europe. This process destroys both spores and pathogens, but minimizes Maillard reactions and maximally maintains the color and taste of the milk [15]. However, not much research has been conducted concerning the impact of UHT processing on milk allergenicity, and this should be further investigated [15].

In egg, most of the allergenic proteins are present in the egg white in comparison to the egg yolk [14]. Still, both fractions had to be present during this study instead of using only the egg white. However, since allergens are also present in the egg yolk, this fraction of the egg also had to be included in this test. Nevertheless, most of the time only the egg white is used [12]. Since egg allergens are very prone to modification through processing, this work used raw freeze-dried egg powder to guarantee maximal allergenicity. Although eggs are mostly used in heated and processed food materials (cookies, pancakes, etc.), few products use minimally heated or raw eggs, such as freshly prepared mayonnaise and chocolate mousse [15]. As shown earlier [16], no effects of freeze-drying on the allergenicity of the eggs could be seen; however, further research should examine this with egg white and yolk.

Furthermore, it is important to exclude other major allergens. For example, in the study by González-Mancebo et al. [12], soy was included in the milk matrices. Also in Protifar^{PLUS}, which has been used in earlier DBPCFC studies as a milk ingredient [10, 11], soy lecithin is used as an emulsifier. However, up to 14 and 60% of patients with IgE-mediated and non-IgE-mediated cow's milk allergy, respectively, might also react to soy [13]. This work used only ingredients which are not listed in EU Regulation No. 1169/2011. Only the cold thickener (waxy maize product) has a precautionary labeling for most of the major food allergens. Due to the small amount added to our powder, possible trace amounts will not influence the test results, because severely allergic IgE patients were excluded from this test. To include more severe allergic patients to this test, appropriate validation of this thickener should be done to exclude possible traces of relevant food allergens.

To assess microbial stability, the a_w values of the cacao powders were determined to predict the growth of bacteria, yeast, and molds over time. All were low enough to inhibit bacterial and mold growth for at least 6 months at ambient temperatures ($a_w \geq 0.91$ and ≥ 0.8 , respectively) [17]. Further, no *E. coli*, *Salmonella* sp., or *B. cereus* species could be detected, which are typical harmful microbial contaminants of milk, egg products, and confectionary products with low a_w [18, 19]. This allows convenient logistics of the matrices because the powder mixes are stable enough to be stored and distributed at ambient temperatures.

The sensory test performed during this work was performed differently from the typical triangle test. During the triangle test, three samples are given at the same moment whereof one sample is different. The objective is to discover whether a difference exists between two samples, no matter what is the attribution of difference [11]. This test is commonly applied during sensory tests of DBPCFC studies [11, 12, 17]. However, the detection of differences during this test will not directly indicate the detection of the allergenic ingredient in the food matrix [12]. Since the placebo and verum samples will be given on separate days during the DBPCFC test, it seemed appropriate to offer the samples sequentially during the taste trials separated by a few minutes without invalidating the test results [12]. Hence, our work focused mainly on the recognition of the allergenic ingredient in the matrix, so the panelists had to answer after every tasting whether the allergenic ingredient was present or not. From the results, no significant differences could be observed. Only for egg was a small difference seen between the panelists who detected eggs in both the verum and placebo matrices. However, the number of panelists who smelled egg was extremely low (10 panelists smelled egg in verum and 3 in placebo samples), so this test lacks a statistically genuine effect. However, this difference must be followed up when performing challenge studies with the egg matrices. Yet, the probability of detecting the allergenic ingredient while consuming the matrices for a longer period could be higher. Therefore, this aspect has to be further evaluated in real DBPCFC situations, which is not commonly done during DBPCFC matrix validation studies.

DBPCFCs at home were conducted using the milk matrices as proof of concept. However, it must be mentioned that home reintroduction/challenges may not be acceptable in patients with severe forms of non-IgE-mediated food allergy [9]. During our work, the patients' allergic history was well determined by the physician (no anaphy-

lactic reactions, no indications for IgE-mediated allergies, reported sign and symptoms, etc.) before starting the at-home challenges. In any case of severe reaction, the test had to be stopped. For example, food protein-induced enterocolitis syndrome (FPIES) is a non-IgE-mediated food allergy characterized by delayed vomiting in infants. This food allergy can be classified into acute and chronic FPIES. Acute reactions are associated with acute, and delayed, repetitive vomiting soon after ingestion, while chronic reactions are associated with more chronic or intermittent vomiting after repeated exposure to the allergens. For severe acute FPIES, these matrices cannot be used at home, but only in a clinical context since peripheral intravenous access is suggested, particularly in patients with a history of severe reactions, emergency department treatment, or hospitalization [20–23]. For mild chronic FPIES reactions, our matrices are well suited at home since the repeated exposure of allergens can then be performed.

Two patients with a positive test outcome showed different symptoms after consuming the verum samples, which were absent during the placebo series (Table 2). When the test outcomes were negative, milk could be reintroduced into the diet. For the patients or parents of the children who did not react, this test was able to reduce their anxiety concerning consuming milk or feeding milk to their children, respectively. However, it is possible that the patients may have a reaction to larger amounts of the allergenic ingredient, so follow-up examination of the patients will still be necessary (e.g., when reintroducing the allergenic ingredient into the diet). For patient 2 for example, no exacerbation was noticed when milk was reintroduced into the diet.

Two children could not finish the total series due to dislike. It is difficult to persuade these young children, who are not able to fully understand the purpose of the test, to continue the DBPCFC. This should be taken into account when interpreting the results, especially when the verum series were not consumed completely. As mentioned in by Vlieg-Boerstra et al. [11], more recipes should be validated properly to cover all age groups and all allergenic foods to also meet the preferences of fussy eaters. During the sensory test, appreciation of the matrices can be assessed, but pilot studies with patients should be conducted in parallel when consuming these matrices for a longer period at home. This is however not performed routinely in the literature when validating novel recipes. Both the adult patients (patients 5 and 6) appreciated the matrices and could not decipher the verum from the placebo. The results of the first DBPCFC tests performed

with the newly developed matrices confirm that these are safe and easy to use in practice, both for children and adults. However, for toddlers, it might be difficult to consume the same portions two periods for 2 weeks, so enough time between two series can be suggested. The use of an easy to fill in evaluation booklet gave the patients the opportunity to register the symptoms and signs they showed during their challenge periods in an easy but detailed way. In the future, however, this could be facilitated by a dedicated, user-friendly app that allows the use of electronic media to register the observed reactions (e.g., smartphone app).

Finally, we would like to address that these matrices can be further implemented to determine IgE-mediated milk and egg allergies. These challenges must be performed in a clinical setting and not at home since more severe allergic reactions are associated with IgE-mediated allergies [9]. However, dilution series have to be made to obtain low-level allergen DBPCFC matrices, which are required to identify the threshold levels of the allergenic ingredient and to assure the safety of the diagnosis method. Validation of the concentrations of the allergenic ingredient in the verum dilutions series will result in a DBPCFC matrix suitable for the diagnosis of IgE-mediated food allergic reactions, as has been described in the work by Cochrane et al. [17] and Vandekerckhove et al. [24].

Conclusion

This work describes the development and evaluation of novel recipes for DBPCFC matrices for milk and egg. Since non-IgE-mediated food allergies are accompanied by delayed food allergic reactions, oral challenges for diagnosis are most often conducted at the homes of the patients with suspected food allergy. Therefore, matrices which allow convenient logistics (stored at ambient temperatures, easy preparation) were developed to facilitate the distribution to clinical centers and the use of these matrices at home. Attention was paid that no other major food allergens were present as ingredients to exclude other food allergic reactions. Through sensory validation, the blinding of the allergenic ingredients could be assessed. A pilot study was conducted with 7 patients to gain more insight concerning the applicability and endurance of consuming these matrices for a longer period of time. None of the patients could tell which were the allergenic verum series. However, more clinical data are needed since 2 young children could not

finish the matrices and challenges with egg were not yet conducted. Negative outcomes provided the respective patients the opportunities to introduce milk back into their diet.

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Statement of Ethics

The study was approved by the Ethics Committee of the University Hospital Ghent, and all patients signed an informed consent to participate and for publication prior to the start of the test.

Disclosure Statement

The authors declare that they have no competing interests.

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Author Contributions

M. Vandekerckhove led the project in total. B. Van Droogenbroeck, M. De Loose, K. Coudijzer, and P. Gevaert made substantial contributions to the conception, design, guidance, and follow-up of the research. B. Van Droogenbroeck was also involved in critically revising the manuscript for important intellectual content. M. Van Winckel and H. Lapeere made substantial contributions to the acquisition, analysis, and interpretation of the data. All authors gave final approval of the version to be published.

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