Development and validation of challenge materials for double-blind, placebo-controlled food challenges in children

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ABSTRACT

Background: The use of double-blind, placebo-controlled food challenges (DBPCFCs) is considered the gold standard for the diagnosis of food allergy. Despite this, materials and methods used in DBPCFCs have not been standardized.

Objective: The purpose of this study was to develop and validate recipes for use in DBPCFCs in children by using allergenic foods, preferably in their usual edible form.

Methods: Recipes containing milk, soy, cooked egg, raw whole egg, peanut, hazelnut, and wheat were developed. For each food, placebo and active test food recipes were developed that met the requirements of acceptable taste, allowance of a challenge dose high enough to elicit reactions in an acceptable volume, optimal matrix ingredients, and good matching of sensory properties of placebo and active test food recipes. Validation was conducted on the basis of sensory tests for difference by using the triangle test and the paired comparison test. Recipes were first tested by volunteers from the hospital staff and subsequently by a professional panel of food tasters in a food laboratory designed for sensory testing. Recipes were considered to be validated if no statistically significant differences were found.

Results: Twenty-seven recipes were developed and found to be valid by the volunteer panel. Of these 27 recipes, 17 could be validated by the professional panel.

Conclusion: Sensory testing with appropriate statistical analysis allows for objective validation of challenge materials. We recommend the use of professional tasters in the setting of a food laboratory for best results.
INTRODUCTION

The use of double-blind, placebo-controlled food challenges (DBPCFCs) has been considered the gold standard for the diagnosis of food allergy for more than a decade.\(^1,2\) Despite this, challenge materials and methods vary from center to center and have not been standardized. There is a need for such standardization to facilitate the use of DBPCFCs in daily practice and to allow for comparison of results obtained in various centers from different parts of the world.\(^3-5\) The challenge materials used at different centers are diverse. Some centers use freeze-dried or concentrated foods masked in capsules or in other foods,\(^1,6-13\) whereas in other studies fresh or native foods masked in other foods are used.\(^1,3,8,14-20\)

Using freeze-dried, heated, or concentrated allergenic foods for DBPCFCs has disadvantages, such as the risk of altered allergenicity.\(^21-24\) Capsules have the further disadvantage that oropharyngeal symptoms are not diagnosed, that early signs of severe anaphylactic reactions might be missed,\(^1,2-4,14,25,26\) and that, depending on the food, large quantities of capsules might need to be consumed. These problems are largely overcome by using allergenic foods in their usual edible form masked in other foods, and this most closely approaches the everyday consumption of such allergenic foods. The major drawbacks of using allergenic foods in their usual edible form are that large amounts of allergenic foods are difficult to disguise.

A number of authors have described the materials or recipes used in DBPCFC studies in which allergenic foods are masked in vehicles consisting of other foods.\(^1,3,8-10,13-20,27-29\) In some of these studies, the exact composition of the recipes used is not clearly or fully documented, and such recipes are thus difficult to implement. Moreover, the methods used to test these materials and validate adequate blinding have not been uniformly rigorous. The purpose of this study was to develop and validate recipes for use in DBPCFCs in children by using allergenic foods, preferably in their usual edible form.\(^3,4,8,14\) Here we describe the test procedure used to validate adequate blinding of the materials developed and the results of this procedure. The results of validation by volunteers are compared with results of validation by professional panelists in a food laboratory.

METHODS

Development of challenge materials (recipes)

Common allergenic foods were selected for which recipes were to be developed and validated for DBPCFCs. These were cow’s milk, heated egg, raw whole egg, raw egg white, soy, peanut, hazelnut, and wheat.\(^2,30\) For each allergenic food, a placebo test food recipe and an active test food recipe was developed. The recipes met the following requirements: (1) acceptable taste; (2) allowance of a
challenge dose high enough to elicit allergic reactions in an acceptable volume (in our experience most young children are able to consume a maximum challenge dose of about 200 mL of liquid challenge material or 50-100 g of solid food within 15 minutes); (3) good matching of sensory properties of placebo and active test food recipes; (4) optimal matrix ingredients, including the avoidance of highly allergenic ingredients for possible use in children allergic to multiple foods; (5) avoidance of the use of frequently suspected foods, such as chocolate; and (6) use of as few ingredients as possible to make recipes acceptable for most patients and to minimize unknown side effects of the ingredients used.\textsuperscript{31} We masked protein equivalent amounts of allergenic foods, which would allow us to compare dose-response reactivities to different foods.

For young children, we created recipes that preferably used the regular daily food consumed by the child because of greater acceptance. For older children, we developed a variety of recipes that would be acceptable to even very fussy eaters.\textsuperscript{32}

**Allergenic food ingredients**

The allergenic foods were used, where possible, in their usual edible form: pasteurized skimmed or semiskimmed milk (1.5%) or Protifar Plus (protein enriched cow’s milk powder) for milk recipes; soy milk for soy recipes; heated egg, irradiated raw whole egg, and raw egg white (irradiated with 15 kGy; Gammaster, Ede, the Netherlands) for egg recipes; roasted and ground peanuts for the peanut recipe (The Nut Company, Doetinchem, The Netherlands); raw (unroasted) and locally bought ground blanched hazelnuts for the hazelnut recipe; and both plain flour and whole meal plain flour for the wheat recipe.

**Vehicles and placebos**

For the kind of recipes in which the placebo test food or vehicle consisted of foods used frequently or daily by children (ie, hydrolyzed formulas, soy-based formulas, or milk), exact matching of the placebo test food and the active test food was sought because sensory differences between the placebo and active test foods would be easily recognized by the child. Thus for these recipes, test foods with no perceivable sensory differences of any kind were developed. However, for most recipes, it is extremely difficult to develop placebo and active test foods that are exactly identical. Organoleptic properties of foods might change slightly, and there might be slight differences in conditions when preparing, storing, and transporting the test foods from one occasion to the next. Thus for the remaining recipes, placebo and active test foods were developed that were as similar as possible but in which small differences were acceptable as long as the presence of the allergenic food could not be detected.
Choice of sensory tests for difference
Sensory tests for difference were used to validate the newly developed recipes. It is often stated that placebo and active recipes should be comparable with regard to taste, aspect, odor, and consistency.\textsuperscript{13,33} For recipes based on vehicles consisting of foods used frequently or daily by the child and for which no perceivable sensory differences of any kind between placebo and active test foods could be tolerated, we used the triangle test for validation.\textsuperscript{34,35} The triangle test belongs to the overall difference tests. The objective of the triangle test is to discover whether a perceivable difference exists between 2 samples, no matter which attribute differs between samples. By using the triangle test, panelists were asked to test 3 samples of a recipe of an allergenic food. Two of these samples were either the placebo or active test food, and the remaining sample was the active or placebo test food, respectively. Samples were coded by using 3-digit random numbers derived from a random table.\textsuperscript{34} The 6 possible sample combinations were offered with equal frequency in random order to the panelists. Subjects were asked to identify the odd sample. The triangle test has a forced-choice procedure, requiring panelists to guess the odd sample when the odd sample is not detectable.

For recipes developed in foods not consumed daily by young children, the paired comparison test (or directional difference test) was used.\textsuperscript{34,35} The paired comparison test belongs to the attribute difference tests. The objective of this test is to determine in which way a particular sensory characteristic, which in our study was the taste of the allergenic food,\textsuperscript{34} differs between 2 samples. For the paired comparison test, more food tasters are needed because of random correct responses of 0.5, compared with the triangle test, in which this chance is 0.33. Using the paired comparison test, panelists were asked to test 1 placebo sample and 1 active sample of a recipe. Samples were coded by using 3-digit random numbers derived from a random table.\textsuperscript{34} Panelists were asked to identify the sample containing the allergenic food. The paired comparison test also has a forced-choice procedure, requiring panelists to guess the right answer when the presence of the allergenic food is not detectable.

Study population and validation of challenge materials (recipes)
The difference tests were first conducted by volunteer panelists from the hospital staff. There were no exclusion criteria, except food allergy to any one of the ingredients of the recipes. For the triangle test, 15 to 20 volunteers took part, and for the paired comparison test, 30 to 40 volunteers participated. Liquid foods were offered in an opaque closed cup with a straw inserted through the lid to hide differences in smell and appearance. Solid foods were visible, and not only taste but also appearance and smell were compared. The latter aspects were also included to allow for development of materials not subject to inadvertent unblinding by persons other than the patient involved in the challenge. The volunteers were
allowed to compare samples as often as desired. Samples were tested at either room temperature or cold directly out of the refrigerator.

If the volunteer panel did not detect statistically significant differences between placebo and active samples, the recipes were subsequently tested by professional sensory panelists in a food laboratory (Department of Food and Business, University of Professional Education Groningen). The test room environment in the food laboratory is designed to minimize the subjects’ biases, to maximize their sensitivities, and to eliminate variables unrelated to the products themselves. The area is free of crowding and confusion, as well as being comfortable, quiet, temperature controlled, and, above all, free of odors and noise. For liquid foods, artificial light is used to exclude the influence of differences in color on the response of the subjects. The food laboratory accommodates several sensory evaluation booths in which the panelists conduct difference testing individually. There is no contact between the panelists during the testing. Panelists can neutralize their taste by drinking water or eating neutral crackers between tests (Fig 1).

On the day of the sensory testing, panelists are not allowed to wear perfumes, wear cosmetics, or use alcohol. During the hours preceding the test, they were asked to abstain from coffee and smoking. This is verified by the panel attendant through questioning of the panelists. The professional panelists are paid for their work (10 Euros per test) and are experienced in conducting sensory difference testing. Panelists are excluded from the sessions if they are allergic to one of the ingredients of the recipes tested or if they are ill or convalescing in any way, as
reported by themselves. The panelists have no contact with the investigators and no vested interest in this study or study outcome. In the food laboratory the triangle test was conducted by at least 33 panelists, and the paired comparison test was performed by at least 54 panelists. For the recipe of raw egg white in pudding, only 30 panelists were used because this recipe was developed and validated in an early phase of this study. If there were no statistically significant sensory differences between placebo and active test foods (triangle test) or if the active test food could not be identified statistically significantly more frequently in the active test samples than in the placebo test samples (paired comparison test), the recipe was considered valid.

Statistics
In SPSS software, 10th edition, the binomial exact test was used to test the differences, with a significance level of .05 (1-tailed probability). One-sided testing was used because the outcome of interest was whether the odd or active sample could be detected with significant frequency.

RESULTS

Validated recipes
Recipes validated by the professional sensory panel of the food laboratory (P > .05) are shown in Table I. The ingredients in the recipes are presented in Appendix 1, which can be viewed at the Journal’s Online Repository. Five cow’s milk recipes, 2 soy recipes, 5 cooked egg recipes, and 1 recipe each for raw whole egg, raw whole egg white, peanut, hazelnut, and wheat were validated. For 6 recipes, the triangle test was used, and for 11 recipes, the paired comparison test was used. Altogether, 27 recipes previously validated by the volunteer hospital panelists were tested in the food laboratory. Of these 27 recipes, only the 17 recipes shown could be validated by the professional sensory panel. The panel was able to detect a difference between active and placebo materials in 7 recipes by using the triangle test and to detect the allergenic food in question in 3 recipes by using the paired comparison test, which included skimmed milk in hydrolyzed cow’s or soy milk formulas and previous recipes for rice milk, higher concentrations of Protifar Plus in hydrolyzed cow’s milk formulas, egg in mashed potatoes, and a previous recipe of peanut in cookies.

To analyze the contribution of the larger number of panelists used in the food laboratory to the increased ability to detect the allergen-containing or odd samples, the results were recalculated with the same number of panelists in the food laboratory as had been used in the initial tests with hospital volunteers. Even when panels of the same size were compared, 7 of the 10 recipes that had been decoded by the professional panel remained decoded.
### Table I. Recipes validated by the professional panelists in the food laboratory

<table>
<thead>
<tr>
<th>Recipe</th>
<th>No. of panelists</th>
<th>No. of correct responses (odd sample or active sample)</th>
<th>Critical no. for significance</th>
<th>P values used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk in Neocate Advance</td>
<td>39</td>
<td>11</td>
<td>19</td>
<td>.688 Triangle test</td>
</tr>
<tr>
<td>Milk in Nutramigen</td>
<td>39</td>
<td>18</td>
<td>19</td>
<td>.065 Triangle test</td>
</tr>
<tr>
<td>Milk in Rice Dream</td>
<td>58</td>
<td>24</td>
<td>36</td>
<td>.881 Paired comparison test</td>
</tr>
<tr>
<td>Protifar in N. Pepti 2</td>
<td>60</td>
<td>23</td>
<td>27</td>
<td>.243 Triangle test</td>
</tr>
<tr>
<td>Protifar in N. Pepti 2, higher concentration</td>
<td>60</td>
<td>23</td>
<td>27</td>
<td>.243 Triangle test</td>
</tr>
<tr>
<td>Soy milk in cow’s milk</td>
<td>33</td>
<td>16</td>
<td>17</td>
<td>.051 Triangle test</td>
</tr>
<tr>
<td>Soy milk in Neocate Advance</td>
<td>33</td>
<td>16</td>
<td>17</td>
<td>.051 Triangle test</td>
</tr>
<tr>
<td>Egg in pancake (free of cow’s milk, soy based)</td>
<td>59</td>
<td>26</td>
<td>36</td>
<td>.783 Paired comparison test</td>
</tr>
<tr>
<td>Egg in minced meat</td>
<td>60</td>
<td>20</td>
<td>37</td>
<td>.994 Paired comparison test</td>
</tr>
<tr>
<td>Egg in minced meat (free of cow’s milk, rice milk based)</td>
<td>54</td>
<td>32</td>
<td>34</td>
<td>.110 Paired comparison test</td>
</tr>
<tr>
<td>Egg in soy custard</td>
<td>56</td>
<td>17</td>
<td>35</td>
<td>.975 Paired comparison test</td>
</tr>
<tr>
<td>Raw whole egg in fruit puree</td>
<td>56</td>
<td>5</td>
<td>35</td>
<td>1.000 Paired comparison test</td>
</tr>
<tr>
<td>Raw whole egg white in pudding</td>
<td>30</td>
<td>16</td>
<td>20</td>
<td>.428 Paired comparison test</td>
</tr>
<tr>
<td>Peanut in cookies</td>
<td>56</td>
<td>27</td>
<td>35</td>
<td>.553 Paired comparison test</td>
</tr>
<tr>
<td>Hazelnut in cookies</td>
<td>56</td>
<td>21</td>
<td>35</td>
<td>.959 Paired comparison test</td>
</tr>
<tr>
<td>Wheat in minced meat</td>
<td>56</td>
<td>13</td>
<td>35</td>
<td>1.000 Paired comparison test</td>
</tr>
</tbody>
</table>

**Amount of allergenic food and volume of highest challenge dose**

In Table II the amount of allergenic food that could be masked in the highest challenge dose is shown. The volume of the highest challenge dose is determined by what we thought to be an acceptable volume for one challenge dose and the amount of vehicle necessary to mask the allergenic food. By varying the challenge volume, the challenge dose can be increased or decreased.

For all recipes, except for peanut and hazelnut, we could mask protein equivalent amounts of allergenic food protein (1.75 g) in the maximum challenge dose. The highest challenge doses of peanut and hazelnut were 1.2 g of peanut (approximately 0.35 g of protein) and 2.5 g of hazelnut (approximately 0.35 g of protein), which
**Table II.** Amount of allergenic food and volume or weight of highest challenge dose of test food recipes

<table>
<thead>
<tr>
<th>Recipe</th>
<th>Amount of allergenic food in highest challenge dose</th>
<th>Volume or weight of highest challenge dose</th>
<th>Protein equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk recipes</td>
<td>50 mL</td>
<td>200 mL drink</td>
<td>1.75 g</td>
</tr>
<tr>
<td>Protifar recipes</td>
<td>2 g</td>
<td>200 mL drink</td>
<td>1.75 g</td>
</tr>
<tr>
<td></td>
<td>2.9 g</td>
<td>200 mL drink</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Soy milk recipes</td>
<td>50 mL</td>
<td>220 to 230 mL drink</td>
<td>1.75 g</td>
</tr>
<tr>
<td>Cooked egg recipes</td>
<td>13.5 g (1/3 egg)</td>
<td>1 small pancake</td>
<td>1.75 g</td>
</tr>
<tr>
<td></td>
<td>13.5 g</td>
<td>1 small meat ball</td>
<td>1.75 g</td>
</tr>
<tr>
<td></td>
<td>13.5 g</td>
<td>280 mL of custard</td>
<td>1.75 g</td>
</tr>
<tr>
<td>Raw egg recipes</td>
<td>13.5 g (whole egg)</td>
<td>445 mL fruit puree</td>
<td>1.75 g</td>
</tr>
<tr>
<td></td>
<td>30 g (egg white)</td>
<td>230 g of pudding</td>
<td>3.3 g</td>
</tr>
<tr>
<td>Peanut recipe</td>
<td>1.2 g (3 peanuts)</td>
<td>55 g of cookies</td>
<td>0.35 g</td>
</tr>
<tr>
<td>Hazelnut recipe</td>
<td>2.5 g (21/2 hazelnuts)</td>
<td>55 g of cookies</td>
<td>0.35 g</td>
</tr>
<tr>
<td>Wheat recipe</td>
<td>17.5 g</td>
<td>55 g of minced meat</td>
<td>1.75 g</td>
</tr>
</tbody>
</table>

were less than the protein equivalent amounts of the other allergenic foods. For Protifar and raw whole egg white, we could mask even greater amounts of allergic food protein (2.5 g of milk protein and 3.3 g of raw whole egg white protein, respectively).

**DISCUSSION**

A number of recipes have been developed over the years for carrying out DBPCFCs.\textsuperscript{1,3,8,10,13-20,27,29} However, the adequacy of the blinding achieved with the use of these challenge materials has not been formally studied. In fact, many authors do not describe the validation procedures or describe a procedure similar to the first step in our protocol by using hospital volunteers.\textsuperscript{15,19,27,36} Even though this was done by us in a rigorous manner, including coding of samples, offering all possible sample combinations with equal frequency in random order, and statistical analysis, a significant number of the recipes validated by the hospital volunteers could be decoded by the professional panel in subsequent testing in the food laboratory (10/27 recipes). Furthermore, many of the published recipes contain concentrations of allergenic foods that are higher than the concentrations we were able to validate\textsuperscript{3,9,10,13,15,16,19,20,28,29}. We tried to validate recipes containing higher concentrations of milk, soy milk, raw egg, peanut, and hazelnut, but they were decoded by the hospital volunteer panel. Taken together, these observations suggest that the validity of challenge materials used for DBPCFCs in some centers might be overestimated, as has also been suggested by others.\textsuperscript{15}
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It is generally stated that if up to 10 g of freeze-dried food or 60 to 100 mL or grams of wet or native food is tolerated in children\textsuperscript{1,11,12,30} or up to 15 g in adults,\textsuperscript{6,25} the allergenic food should be tolerated. However, the conversion factor from dried to wet foods and the exact nature of the food source used has not been clearly documented, and the conversion requires some important assumptions.\textsuperscript{5} In the literature the challenge materials used might be expressed in total amounts of allergenic food or in the amount of protein contained in allergenic foods, whereas in other cases assumptions must be made in expressing the challenge dose in total amount of the challenged food or the amount of protein. Again these differences stress the importance of standardization of challenge materials to allow for better comparison of results of DBPCFCs.\textsuperscript{5}

The difficulty in developing recipes is masking amounts of allergenic food in an acceptable volume that are high enough to elicit reactions.\textsuperscript{25,36} In our study the highest challenge dose was determined on the basis of the maximum amount of allergenic food that could be hidden in what we thought to be an acceptable volume of a single challenge dose for a child (Table II). The challenge procedure in which these materials were used included a 4- to 7-step incremental design in which progressively greater quantities of the same recipe were administered. Our experience in using these recipes for DBPCFCs is that most children are able to consume the highest challenge dose. To date, there are little published data on dose-response relationships in food challenges and the highest dose necessary to avoid false-negative results of DBPCFCs in children.\textsuperscript{12,13} Sicherer et al\textsuperscript{12} administered a cumulative dose of 10 g dry weight of dehydrated food or an equivalent amount of liquid food to patients, with a final dose of 2.5 g of dehydrated milk, egg, soy, wheat, fish, or peanut, and found that 4% of these negative double-blind challenge results was followed by positive open challenge results. One might assume that the dry weight of the final dose is equivalent to about 25 mL of fresh milk or 25 g of whole egg. Thus our highest dose for egg and milk are in the same order of magnitude as that used by Sicherer et al. Schade et al\textsuperscript{13} used Protifar in amounts equivalent to about 45 mL of fresh milk as the highest dose, which is quite close to our maximum dose for milk and soy. Schade et al reported no false-negative immediate reactions. We have also encountered no false-negative immediate allergic reactions in the more than 90 DBPCFCs we have carried out to date.

In the literature different kinds of challenge materials are used in DBPCFCs.\textsuperscript{5} Sometimes freeze-dried foods are used, and sometimes native or fresh foods in their usual edible form are used. For egg, most investigators used egg white, and some used whole egg, usually raw but sometimes cooked. For milk, many different foods are used: fresh nonfat or semiskimmed milk, nonfat milk powder, or infant formulas are used. For peanut, ground peanuts are usually used, but peanut butter or peanut flour has also been used.\textsuperscript{5} For hazelnuts, some investigators use
raw hazelnuts, whereas in other studies it remains unclear whether raw or roasted hazelnuts are used.\textsuperscript{16,20} The use of these different challenge materials complicates the comparison of recipes used and the results obtained.\textsuperscript{5} We decided to use allergenic foods in their usual edible form because this would best mimic real-life exposure. Fresh foods are easily available and relatively easy to prepare. This was possible for all recipes, except for the milk recipes in whey hydrolysates, in which Protifar Plus was used. Details about the allergenicity of Protifar Plus are not available. Schade et al\textsuperscript{13} used Protifar for DBPCFCs in children and did not observe false-negative reactions, suggesting that the allergenicity of this product has not been reduced in comparison with that of pasteurized milk.

In general, the power of sensory tests for difference is poor. Unattainably large numbers of panelists are needed for high power, depending on the true proportion of panelists able to detect a difference that is considered acceptable.\textsuperscript{34,35} For example, for a triangle test with a power of 80\%, an $\alpha$ value of .05, a $\beta$ value of .2, and a true proportion of panelists able to detect a difference of 10\%, 325 panelists are required. For the paired comparison test, the panel size is even larger. Thus it is important to increase power by performing sensory testing under optimal conditions by using a professional panel in a food laboratory designed for sensory testing to maximize the panelists’ sensory sensitivity. Our results show that sensory testing by professional panelists in a food laboratory has greater power than sensory testing by volunteers. In conclusion, we recommend the use of standardized challenge materials in DBPCFCs. Sensory testing with appropriate statistical analysis allows for objective validation of challenge materials. We recommend the use of professional tasters in the setting of a food laboratory for best results. More work needs to be done on the maximum dose to be used considering the age of the patients and the preparation of the allergenic food used (eg, raw, heated, and freeze-dried).\textsuperscript{12,37} To eliminate possible false-negative test results caused by these or other factors, DBPCFCs should always be followed by an open challenge or introduction until the allergenic food is consumed in a meal size portion or in amounts in which it is normally used by the child.

We thank Quest International for their kind supply of peanut and hazelnut flavors and the Nut Company for their supply of ground peanuts in collaboration with the American Peanut Council. We also thank Professor R. Aalberse (Amsterdam, The Netherlands) for his comments on the statistical analysis of our results.
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References

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APPENDIX: INGREDIENTS OF VALIDATED RECIPES

Ingredients are listed in the order in which they are incorporated in the recipe.

1. MILK IN NEOCATE ADVANCE $E_{1-6}$
   **Active test food**
   - 150 ml of Neocate Advance $E_{8, E_{12}}$
   - (50 g of powder + 128 ml water)
   - 50 ml pasteurised skimmed milk

   **Placebo test food**
   - 200 ml of Neocate Advance $E_{8, E_{12}}$
   - (50 g of powder + 170 ml water)

2. MILK IN NUTRAMIGEN $E_{1-6}$
   **Active test food**
   - 150 ml of Nutramigen $E_{9, E_{13}}$
   - (27 g of powder + 135 ml water)
   - 50 ml pasteurised skimmed milk

   **Placebo test food**
   - 200 ml of Nutramigen $E_{9, E_{13}}$
   - (27 g of powder + 180 ml water)

3. MILK IN RICE DREAM $E_{1-5}$
   **Active test food**
   - 130 ml of rice milk with added calcium
   - (Rice Dream) $E_{14}$
   - 50 ml of pasteurised skimmed milk
   - 6 g of ready-to-eat rice flour (Nutrix) $E_{15}$
   - 20 ml of fruit syrup (grenadine)

   **Placebo test food**
   - 180 ml of rice milk with added calcium
   - (Rice Dream) $E_{14}$
   - 3 g of dairy free margarine
   - 6 g of ready-to-eat rice flour (Nutrix) $E_{15}$
   - 20 ml of fruit syrup (grenadine)

4. PROTIFAR PLUS IN NUTRILON PEPTI 2 $E_{1-6}$
   **Active test food**
   - 200 ml of Nutrilon Pepti 2 $E_{10, E_{15}}$
   - (27 g of powder + 180 ml water)
   - 2 g of Protifar Plus $E_{11, E_{15}}$

   **Placebo test food**
   - 200 ml of Nutrilon Pepti 2 $E_{10, E_{15}}$
   - (27 g of powder + 180 ml water)

5. PROTIFAR PLUS IN NUTRILON PEPTI 2 $E_{1-6}$
   (HIGHER CONCENTRATION)
   **Active test food**
   - 200 ml of Nutrilon Pepti 2 $E_{10, E_{15}}$
   - (27 g of powder + 180 ml water)
   - 2,9 g of Protifar Plus $E_{11, E_{15}}$

   **Placebo test food**
   - 200 ml of Nutrilon Pepti 2 $E_{10, E_{15}}$
   - (27 g of powder + 180 ml water)

6. SOY MILK IN NEOCATE ADVANCE $E_{1, E_{3-7}}$
   **Active test food**
   - 150 ml of Neocate Advance $E_{8, E_{12}}$
   - (50 g of powder + 128 ml water)
   - 50 ml of soy milk with no sugar or salt (Alpro) $E_{16}$
   - 6 g of ready-to-eat rice flour (Nutrix) $E_{15}$
   - 15 ml of fruit syrup (grenadine)

   **Placebo test food**
   - 200 ml of Neocate Advance $E_{8, E_{12}}$
   - (50 g of powder + 170 ml water)
   - 3 g of dairy-free margarine
   - 6 g of ready-to-eat rice flour (Nutrix) $E_{15}$
   - 15 ml of fruit syrup (grenadine)
7. SOY MILK IN MILK E1, E3-6

<table>
<thead>
<tr>
<th>Active test food</th>
<th>Placebo test food</th>
</tr>
</thead>
<tbody>
<tr>
<td>145 ml of semi skimmed milk (1.5%)</td>
<td>185 ml of semi skimmed milk (1.5%)</td>
</tr>
<tr>
<td>50 ml of soy milk with no sugar or salt (Alpro)*16</td>
<td>--</td>
</tr>
<tr>
<td>20 ml of unwhipped cream (35 %)</td>
<td>30 ml of unwhipped cream (35 %)</td>
</tr>
<tr>
<td>15 ml of fruit syrup (grenadine)</td>
<td>15 ml of fruit syrup (grenadine)</td>
</tr>
</tbody>
</table>

8. EGG IN PANCAKE E2-4

<table>
<thead>
<tr>
<th>Active test food</th>
<th>Placebo test food</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 g of wheat flour</td>
<td>26 g of wheat flour</td>
</tr>
<tr>
<td>53 ml of semi skimmed milk (1.5 %)</td>
<td>66 ml of semi skimmed milk (1.5 %)</td>
</tr>
<tr>
<td>0.8 g of dried yeast</td>
<td>0.8 g of dried yeast</td>
</tr>
<tr>
<td>13.5 g of lightly beaten egg</td>
<td>-</td>
</tr>
<tr>
<td>0.2 g of salt</td>
<td>0.2 g of salt</td>
</tr>
<tr>
<td>12 g of grated apple</td>
<td>12 g of grated apple</td>
</tr>
<tr>
<td>8 g of dairy free margarine (for baking)</td>
<td>8 g of dairy free margarine (for baking)</td>
</tr>
<tr>
<td>4.5 g of castor sugar</td>
<td>4.5 g of castor sugar</td>
</tr>
</tbody>
</table>

9. EGG IN PANCAKE -SOY BASED E3, E4, E7

<table>
<thead>
<tr>
<th>Active test food</th>
<th>Placebo test food</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 g of wheat flour</td>
<td>26 g wheat flour</td>
</tr>
<tr>
<td>53 ml of soy milk with no sugar or salt (Alpro)*16</td>
<td>66 ml soy milk with no sugar or salt (Alpro)*16</td>
</tr>
<tr>
<td>1.5 g of soy cream (Soy Cuisine, Alpro)*16</td>
<td>4 ml of soy cream (Soy Cuisine, Alpro)*16</td>
</tr>
<tr>
<td>0.8 g of dried yeast</td>
<td>0.8 g of dried yeast</td>
</tr>
<tr>
<td>13.5 g of lightly beaten egg</td>
<td>-</td>
</tr>
<tr>
<td>0.2 g of salt</td>
<td>0.2 g of salt</td>
</tr>
<tr>
<td>16 g of grated apple</td>
<td>16 g of grated apple</td>
</tr>
<tr>
<td>8 g of dairy free margarine (for baking)</td>
<td>8 g of dairy free margarine (for baking)</td>
</tr>
<tr>
<td>4.5 g of castor sugar</td>
<td>4.5 g of castor sugar</td>
</tr>
</tbody>
</table>

10. EGG IN MINCED MEAT E2-4

<table>
<thead>
<tr>
<th>Active test food</th>
<th>Placebo test food</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 g of minced beef</td>
<td>40 g of minced beef</td>
</tr>
<tr>
<td>13.5 g of lightly beaten egg</td>
<td>14 ml of semi skimmed milk (1.5 %)</td>
</tr>
<tr>
<td>6.5 g of wheat flour</td>
<td>6.5 g of wheat flour</td>
</tr>
<tr>
<td>6.5 g of bread crumbs (egg free)</td>
<td>6.5 g of bread crumbs (egg free)</td>
</tr>
<tr>
<td>0.2 g of salt</td>
<td>0.2 g of salt</td>
</tr>
<tr>
<td>0.2 g of pepper</td>
<td>0.2 g of pepper</td>
</tr>
<tr>
<td>8 g of dairy free margarine (for baking)</td>
<td>8 g of dairy free margarine (for baking)</td>
</tr>
</tbody>
</table>
### 11. EGG IN MINCED MEAT – RICE MILK BASED E2-4, E7

**Active test food**
- 40 g of minced beef
- 13,5 g of lightly beaten egg
- 6,5 g of wheat flour
- 6,5 g of bread crumbs (egg and dairy free)
- 0,2 g of salt
- 0,2 g of pepper
- 8 g of dairy-free margarine (for baking)

**Placebo test food**
- 40 g of minced beef
- 14 ml of rice milk with added calcium (Rice Dream) E14
- 6,5 g of wheat flour
- 6,5 g of bread crumbs (egg and dairy free)
- 0,2 g of salt
- 0,2 g of pepper
- 8 g of dairy-free margarine (for baking)

### 12. EGG IN SOY CUSTARD E3-7

**Active test food**
- 200 ml of soy custard (vanilla, Alpro) E16
- 67 ml of red grape juice
- 13,5 g of hard boiled and mashed egg

**Placebo test food**
- 200 ml of soy custard (vanilla, Alpro) E16
- 57 ml of red grape juice
- 5 g of soy cream (Soy Cuisine, Alpro) E16

### 13. RAW WHOLE EGG IN FRUIT PUREE E3, E4, E7

**Active test food**
- 300 g of fruit puree (Olvart, nr. 307) E15
- 120 ml of orange juice
- 12 g of soy cream (Soy Cuisine, Alpro) E16
- 13,5 g of raw lightly beaten egg

**Placebo test food**
- 300 g of fruit puree (Olvart, nr. 307) E15
- 36 ml of orange juice
- 24 g of soy cream (Soy Cuisine, Alpro) E16
- --

### 14. RAW WHOLE EGG WHITE IN PUDDING E3-6

**Active test food**
- 200 g of prepared vanilla pudding (Saroma) E17
- (semi-skimmed milk (1,5 %) and blancmange powder)
- 30 g of raw lightly beaten egg white

**Placebo test food**
- 230 g of prepared vanilla pudding (Saroma) E17
- (semi-skimmed milk (1,5%) and blancmange powder)
- --

### 15. PEANUT IN COOKIES E1, E2, E4, E7

**Active test food**
- 8,5 g of whole wheat flour
- 8,5 g of wheat flour
- 3 g of wheat germ
- 15 g of cane sugar
- 15 g of dairy-free margarine
- 0,3 of salt
- 6 g of desiccated coconut
- 1,2 g of roasted, ground peanuts
- 0,03 ml of hazelnut flavour QL 13849 E18

**Placebo test food**
- 8,5 g of whole wheat flour
- 8,5 g of wheat flour
- 3 g of wheat germ
- 15 g of cane sugar
- 15 g of dairy-free margarine
- 0,3 g of salt
- 6 g of desiccated coconut
- 0,03 ml of peanut flavour QL 35189 E18
- 0,03 ml of hazelnut flavour QL 13849 E18
16. HAZELNUT IN COOKIES E1-3, E7

**Active test food**
- 8,5 g of whole wheat flour
- 8,5 g of wheat flour
- 3 g of wheat germ
- 15 g of cane sugar
- 15 g of dairy-free margarine
- 0,3 of salt
- 6 g of desiccated coconut
- 2,5 of g ground blanched unroasted hazelnuts

**Placebo test food**
- 8,5 g of whole wheat flour
- 8,5 g of wheat flour
- 3 g of wheat germ
- 15 g of cane sugar
- 15 g of dairy-free margarine
- 0,3 g of salt
- 6 g of desiccated coconut
- 0,045 ml of hazelnut flavour QL 13849 E18

17. WHEAT IN MINCED MEAT E1-4, E7

**Active test food**
- 60 g of minced beef
- 16 ml of rice milk with added calcium (Rice Dream) E14
- 5,5 g of whole wheat flour
- 12 g of wheat flour
- 0,3 g of salt
- 0,3 g of pepper
- 16 g of dairy-free margarine for baking

**Placebo test food**
- 60 g of minced beef
- 24 ml of rice milk with added calcium (Rice dream) E14
- 5,5 g of whole buckwheat flour
- 12 g of whole rice flour
- 0,3 g of salt
- 0,3 g of pepper
- 16 g of dairy-free margarine for baking

**Characteristics of recipes:**
- E1: Recipe contains no egg
- E2: Recipe contains no soy or soy lecithin
- E3: Recipe contains no peanut or peanut oil
- E4: Recipe contains no nuts or nut oil
- E5: Recipe contains no wheat
- E6: Recipe contains no gluten
- E7: Recipe contains no cow’s milk

**Product type:**
- E8: Amino acid-based infant formula
- E9: Intensively hydrolysed casein formula
- E10: Intensively hydrolysed whey formula
- E11: Protein-enriched cow’s milk powder (comparable to Resource Instant protein, Novartis)

**Manufacturers:**
- E12: SHS International Ltd, UK
- E13: Mead Johnson, Division of Bristol-Myers Squibb, Avansville USA
- E14: Imagine foods Ltd, London, UK
- E15: Nutricia/Numico, Zoetermeer, The Netherlands
- E16: N.V. Vandemoortele, Roosendaal, The Netherlands
- E17: Honig, Koog a/d Zaan, The Netherlands
- E18: Internatio Möller, Mechelen, Belgium