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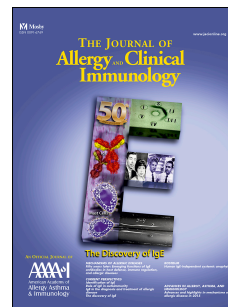| Title	Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in children with peanut allergy
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Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in peanut allergic children

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1 **Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge**
2 **study to validate eliciting doses in peanut allergic children**

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40

45   **Abbreviations**

46   DBPCFC   Double blind, placebo-controlled food challenge

47   ED   Eliciting dose

48   FAQLQ   Food allergy related quality of life questionnaire

49   FEV_1 Forced expiratory volume in 1 second

50   LOAEL   Lowest adverse effect level

51   OFC   Oral food challenge

52   PA   Peanut allergy

53   PAL   Precautionary allergen labelling

54   PATS   Peanut allergen threshold study

55   spIgE   Specific IgE

56   SPT   Skin prick test

57   VITAL   Voluntary Incidental Trace Allergen Labelling

58   VSEP   VITAL scientific expert panel

59
60   **Capsule summary**
61   The derived ED_{05} for peanut (1.5mg peanut protein) was given in a single dose to 378 peanut
62   allergic subjects. Only 8 subjects (2.1%) met predetermined criteria for an objective reaction,
63   suggesting the derived ED_{05} could be used as a safe reference dose.
64
65   **Clinical Implications**
66   The ED_{05} for peanut (1.5mg peanut protein) was validated in a multicentre study, using a novel
67   single dose challenge design, which provides a significant quality of life benefit for parents of
68   participants and could be adapted to other research or clinical settings.
69
70   **Keywords**
71   Eliciting dose (ED), Food Allergy related Quality of Life Questionnaires-(FAQLQ), Single dose,
72   Peanut thresholds**,** Oral Food Challenges (OFC), Voluntary Incidental Trace Allergen Labelling
73   (VITAL). Peanut Allergen Threshold Study (PATS)
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Abstract

Background

Eliciting doses (ED) of allergenic foods can be defined by the distribution of threshold doses for individuals within a specific population. ED_{05} is the dose that elicits a reaction in 5% of allergic subjects. The predicted ED_{05} for peanut (PN) is 1.5 mg of peanut protein (6 mg whole peanut).

Objective

We sought to validate the predicted peanut ED_{05} (1.5 mg) with a novel single dose challenge.

Methods

Consecutive eligible peanut allergic children in 3 centres were prospectively invited to participate, irrespective of previous reaction severity. Predetermined criteria for objective reactions were used to identify ED_{05} single dose reactors.

Results

518 children (mean age 6.8 years) were eligible. No significant demographic or clinical differences were identified between 381(74%) participants and 137 (26%) non-participants or between subjects recruited at each centre. 378 children (206 male) completed the study. Almost half the group reported ignoring precautionary allergen labelling. 245 (65%) experienced no reaction to the single dose of peanut. 67 (18%) reported a subjective reaction without objective findings. 58 (15%) experienced signs of a mild and transient nature that did not meet the pre-determined criteria. Only 8 subjects (2.1%, 95% CI 0.6%-3.4%) met the pre-determined criteria for an objective and likely related event. No child experienced more than a mild reaction, 4 of the 8 received oral antihistamines only and none received epinephrine. Food allergy related quality of life improved from baseline to 1 month post challenge regardless of outcome (eta squared = 0.2, p <0.0001). Peanut SPT, peanut and Ara h 2 spIgE levels were not associated with objective reactivity to PN ED_{05}.

105   **Conclusion**

106   A single administration of 1.5 mg PN protein elicited objective reactions in fewer than the predicted

107   5% of peanut-allergic subjects. The novel single dose OFC appears clinically safe and patient-

108   acceptable, regardless of the outcome. It identifies the most highly dose-sensitive food allergic

109   population, not otherwise identifiable using routinely available peanut SPT or spIgE levels but this

110   single-dose approach has not yet been validated for risk assessment of individual patients.

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130 **Introduction**

131 Food allergic individuals are clinically selected to participate in diagnostic or research oral food

132 challenge (OFC) protocols that use graded, incremental doses administered at short, fixed time

133 intervals. Subjects who have experienced anaphylaxis are often not offered routine clinical OFC

134 and may be excluded from research OFC protocols (1). It is generally not possible from graded

135 protocols to determine whether a reaction has occurred to a *discrete* threshold dose of the allergenic

136 food or alternatively has been the result of the *cumulative* dose consumed by the allergic individual

137 at the time of reaction.

138

139 The eliciting dose (ED) for a peanut allergic reaction in 5% of the peanut allergic population (ED_{05})

140 has been estimated at 1.5 mg of peanut protein (6 mg of whole peanut) based upon the population

141 distribution of threshold doses (children and adults) from graded, blinded oral challenges of 750

142 peanut allergic individuals (2-4).

143

144 This study aims to assess the precision of the predicted ED_{05} using a single dose challenge (6 mg

145 peanut = 1.5mg of peanut protein, approximately $1/100^{th}$ of a peanut kernel) in an unselected group

146 of peanut-allergic children and to validate the processes used to develop the only existing reference

147 doses for peanut, which have been based upon the ED_{01} (which is the dose which elicits reactions in

148 1% of subjects studied) (2). It is likely that subjects who react only mildly at the ED_{05} would

149 tolerate the ED_{01} at least as well (4). This may assist clinicians, regulators and other stakeholders in

150 risk management for peanut allergic subjects.

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155 **Methods**

156 We have already published an in-depth description of the background and methodology of the

157 PATS study (please see reference 5). Additional details are provided below.

158

159 **Recruitment**

160 This multi-centre study involved three geographically diverse teaching centres, set in University-

161 affiliated hospitals, providing local, regional and national allergy services. To minimise recruitment

162 bias, the protocol required that the study was discussed fully with every potentially suitable child

163 and family, met during routine medical encounters in clinic or hospital attendances. Families who

164 chose not to participate were asked to complete a study-specific "non-participant" questionnaire,

165 adapted from Osborne et al (6) and to give written informed consent for their routinely available

166 laboratory data to be examined anonymously in the study. Inclusion and exclusion criteria are

167 shown in Table 1.

168

169 **Food Allergy related Quality of Life Questionnaires-(FAQLQ)**

170 Validated FAQL-Parental form (FAQL-PF) and FAQL Child form (FAQL-CF) questionnaires were

171 self-administered prior to OFC (T1) and 1 month after OFC (T2) to assess the impact of this novel

172 single dose OFC protocol on FAQL (8). FAQL-PF and CF are age appropriate questionnaires that

173 assess the health related quality of life (HRQL) of children with food allergy. The PF version is

174 completed by a parent of the food allergic child (0-12 years) and the CF by the child themselves (8-

175 12 years) on a 7-point scale ranging from not at all (1) to extremely (7). It has been found to have

176 excellent reliability ($\alpha > 0.9$), and construct, cross-cultural, content and longitudinal validity. A

177 higher score on either questionnaire reflects higher burden and poorer FAQL. A lower score

178 reflects lower burden/better FAQL.

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Single dose Oral Food Challenge (OFC)

The shelf-stable single-dose challenge cookies were manufactured at University of Nebraska-

Lincoln, USA and then distributed to participating clinic centres. Peanut content was determined

using the Neogen ® Veratox ® Quantitative Peanut Allergen Test (Neogen Corporation, Lansing

MI). This assay was also used to establish a validated mixing method to achieve a homogeneous

incorporation of peanut flour into the formulation as well as determining that all ingredients in the

formulation were below the limit of quantitation (2.5 ppm). The stability of product was established

by meeting acceptable criteria for water activity and microbial load. To maintain taste and texture

cookies were stored frozen until use. The single-dose cookie (6 mg whole peanut = 1.5 mg peanut

protein) consisted of granulated sugar, brown sugar, all-purpose wheat flour, vegetable shortening,

salt, baking soda and light roast, partially defatted peanut flour (Golden Peanut Company,

Alpharetta, Georgia USA). The cookie was eaten under standard open OFC conditions in hospital.

For subjects allergic to other cookie ingredients (e.g. wheat), the peanut dose of 1.5 mg peanut

protein was administered as the same light roast, partially defatted peanut flour in a vehicle food of

the subject's choice. Routine OFC monitoring was performed, according to local clinical practice.

Children were observed until 2 hours after OFC if no symptoms and signs were elicited or until 2

hours after such symptoms and signs had resolved, with or without treatment.

Criteria for a positive OFC result.

A highly liberal, inclusive strategy was used to capture clinical data during the OFC. Staff were

encouraged to make extensive notes, recording *any* physical or behavioural changes observed or

self-reported during the single-dose OFC. Predetermined objective criteria were used because the

ED_{05} was predicted on the basis of challenge-associated objective responses only (1-4). The prior

agreed upon objective criteria for a positive OFC result occurring within 2 hours of ingestion were:

205 3 or more concurrent noncontact urticaria persisting for at least 5 minutes; or perioral or periorbital

206 angioedema; or rhinoconjunctivitis including sneezing; or diarrhoea; or vomiting (excluding gag

207 reflex); or anaphylaxis (with evidence of circulatory or respiratory compromise, e.g. persistent

208 cough, wheeze, change in voice, stridor, difficulty breathing, and collapse) (9).

209 Subjective symptoms were also recorded, such as: palatal itch, headache, dizziness, bloating,

210 abdominal pain, cramps, muscle aches, aching joints, anxiety, tension, and agitation.

211

212 **Case definition**

213 When the clinical study was completed all co-investigators met in person and reviewed all clinical

214 comments written by staff in each centre during the study. The above criteria were applied and

215 cases were designated "objective" or "subjective" and then as having met or not met the

216 predetermined objective criteria as above.

217

218 **Blood test**

219 A blood sample was taken for peanut spIgE component analysis (local hospital laboratories, using

220 ThermoFisher Immunocaps®, according to manufacturer's instructions) and quantitative peanut-

221 specific IgE fluoroenzyme immunoassays 20 minutes after OFC.

222

223 **Sample size estimation**

224 Assuming that the observed proportion of the sample that react to the single dose OFC is 5%, a

225 sample size of 375 corresponds to a 95% confidence interval for the population proportion with a

226 lower limit of 3.1% and an upper limit of 7.8% using the properties of the binomial distribution.

227 The investigators felt that this degree of precision in estimation was sufficient to rule out gross

228 incompatibility between the predicted and observed proportion of participants reacting to the single

229 dose.

230   **Statistics**

231   Data were analysed using SPSS Version 22(IBM, Evanston, Illinois, USA). Two sample t-tests for

232   continuously valued variables and Pearson's chi- square test or Fisher's exact test (for low

233   prevalences) for binary variables were conducted to determine the extent of any covariate

234   imbalance between participants and non-participants. Differences in means and proportions

235   between centres were also examined using similar statistical methods. The impact of the single dose

236   protocol on FAQL was analysed using multivariable regression analysis.

237   Partial Eta-squared' (η_p^2), also known as 'R-Squared', was the effect size produced by the

238   statistical tests used in this study. There are many advantages to including effect size when

239   reporting significant results. Effect size is not influenced by sample size or number of variables.

240   While a significant result (p value) shows whether an effect exists, it does not reflect the size of the

241   effect. Therefore both the magnitude (effect size) and significance (P value) are essential results to

242   be reported (10-12). A small effect is less than 0.08, a medium effect is less than 0.24 and a large

243   effect size is 0.25 and above (11).

244

245   *Ethical approval*

246   This Study was approved by Cork University Hospital Research Ethics Committee (ECM 4 g),

247   Melbourne Royal Children's Hospital Human Research Ethics Committee (HRECApp 32166A),

248   and the Partners Human Research Committee (2012P002475). Written, informed parental and

249   adolescent consent and younger children's assent (according to local IRB age-related

250   requirements) were obtained.

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254   **Results**

255   Between October 2013 and February 2015, 518 patients were serially approached for participation

256   (Figure). One hundred thirty-seven individuals were deemed either ineligible or did not wish to take

257   part in the study. Three hundred seventy-eight completed the challenge protocol. Three subjects did

258   not complete the protocol. Comparisons of participants and non-participants in each centre are

259   shown in Table 2. Univariate analysis of variance showed no significant age differences between

260   participants and non-participants (p = 0.62), controlling for centre location (p=0.84,). Sixty

261   percent of the overall sample was male. Twenty-two percent of females approached did not

262   participate, compared to 30% of males (x^2= 6.7, p=0.035). There was no difference in participant

263   sex between centres (x^2= 2.6, p=0.63).

264

265   A significant association was found between entry criteria and study centre location. Twenty-seven

266   percent of Irish subjects had been diagnosed with peanut allergy by the most stringent criterion

267   (positive OFC), compared to 11% in Australia and only 2.5% in the US, (p<0.001). However,

268   diagnostic method did not significantly differ between participants and non-participants (x^2= 3.6,

269   p=0.17) or between sexes (x^2= 6.17, p=0.19).

270

271   **Reactions to single dose ED_{05} OFC**

272   381 participants took part in this stage of the study; two were excluded due to incomplete ingestion

273   of the peanut cookie. One subject was excluded before starting the protocol due to inter-current

274   illness, evident on clinical examination on the day of study. 378 subjects completed the protocol.

275   362 subjects (96%) received the single dose in the cookie. The remaining 16 subjects received

276   peanut flour instead in another vehicle food of their choice.   There were no significant differences

277   in reaction type between the 362 children who ate the standard cookie and the 16 children who ate

278   the peanut flour in another vehicle (x^2= 2.21, p=0.53).

279

280   245 subjects showed no reaction to the cookie single dose OFC (Table 3). For 133 subjects, some

281   comment indicative of a possible reaction was recorded in the written OFC records. Sixty-seven

282   reported subjective symptoms only. Sixty-six events were considered objective, but 58 of these did

283   not meet the predetermined criteria. The very mild and transient objective symptoms that did not

284   meet the predetermined criteria included non-persistent usually single sneeze, non-persistent

285   usually single cough, small areas of transient erythema, and fewer than 3 hives lasting <5 minutes.

286   Eight participants experienced objective events that met the predetermined criteria (Table 4). All

287   eight subjects who met the pre-determined criteria consumed the cookie not an alternative vehicle.

288   No participant experienced more than a mild reaction; four of the 8 most objectively reacting

289   subjects were treated with oral antihistamines. No other subject was treated and none received

290   epinephrine.

291

292   Multivariable regression analysis showed no significant differences for age and centre, reaction

293   type or participant/ non participant status. The eight subjects who met the predetermined objective

294   criteria were no different in age to others included in the study (Table 4).

295

296   Study centre and reaction type were not significantly related to diagnostic entry criterion (x^2=3.39,

297   p= 0.76). Subjects' sex was not significantly related to reaction type (x^2=4.76, p= 0.19).

298

299   Univariate analyses showed peanut spIgE, Ara h1, Ara h2, Ara h3, Ara h8, Ara h9 spIgE levels and

300   total IgE levels had no effect on inclusion criterion met or participant/non-participant status,

301   (p 0.21 - 0.99) (Table 5). Peanut SPT differed between study centre location ($\eta_p^2 = 0.02$, p = 0.03)

302   with a small effect size (11), but not for reaction type (p=0.25). Irish subjects had the lowest mean

303   wheal size (M= 9.50 mm, SD= 2.66) and Australia the highest (M=15mm, SD=6.47). No other skin

304   or blood tests were significant for either type of reaction or location (p>0.05).

305

306 Adherence to precautionary labelling at study entry was significantly lower in Australia where 76%

307 ignore labelling compared to Ireland (33%) and US (36%) ($x^2 = 66.21$, p<0.001). Proxy and self-

308 reported adherence to precautionary allergen labelling did not significantly change from T1-T2 and

309 was unaffected by age of child, study centre or diagnostic criteria met (p= 0.82-0.42).

310

311 **Food allergy-related quality of life**

312   Baseline scores (before OFC) in the FAQL-PF predicted likelihood of reporting subjective vs

313 objective symptoms (after OFC) (p=0.001). In effect, children who later experienced subjective

314 symptoms to the single dose of peanut had the most adverse impact on FAQL at baseline (Mean

315 =2.6, SD= 1.4). Those who did not experience any reaction had the best FAQL (lowest burden) at

316 baseline (Mean= 1.8, SD=1.3). This provides further evidence of the association between clinical

317 and psychological factors in food allergy.

318

319 There was a significant main effect for time from T1 to T2 for parent reported proxy FAQL-PF (η_p^2

320 =0.24, p=0.014), with a medium to large effect size (11), where parents reported an improvement in

321 FAQL for their children from baseline to 1 month post protocol. There was a significant three-way

322 interaction between age, sex and time ($\eta_p^2 = 0.11$, p=0.014) with a medium effect size (11).

323 Regardless of age or sex of child, parents reported improved FAQL at T2. Younger boys

324 experienced a higher impact, whereas as age increased, parents reported more adverse impact for

325 girls. Diagnostic criteria and type of reaction elicited in the single dose study were not significant.

326

327   Children's self-reported FAQL-CF also improved from baseline (T1) to 1 month post protocol (T2)

328   (η_p^2=0.5, p=0.001) with a very large effect size (11). Again there was no effect on FAQL by

329   inclusion criteria met or type of reaction (p=0.158).

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Discussion

The novel single-dose PATS findings strongly support the safety of the statistically determined ED_{05} based upon population dose-distribution modelling (2) for administration to a non-selected patient population. The protocol was very acceptable to families and was clinically very safe. This approach offers the opportunity to identify the most dose-sensitive peanut allergic population in a safe and efficient manner. It could be adapted for other major allergenic foods.

Population EDs can be estimated by statistical dose-distribution modelling of individual patient threshold doses (2-4). ED estimates can vary depending upon the choice of model. The single-dose PATS approach serves as a useful way to validate the ED estimates and select the best parametric model. In this single-dose PATS, the percentage of patients reacting with the predetermined objective criteria (2.1%) was lower than predicted from the log-normal model (5%; 95% CI of 3.1-7.8%). Several reasons could explain the observed difference between the predicted 5% versus the observed 2.1% rate. First, selection bias toward more highly sensitive patients could have occurred with the 750 peanut-allergic subjects in the modelled dataset as many of the patients included in the dataset were from tertiary allergy clinics which could contribute to a bias toward a more sensitive peanut-allergic population (2,3), though this study group of consecutive patients was also recruited in tertiary centres. Second, although objective responses were used in the clinics conducting threshold challenges and the PATS, the objective criteria used to establish the lowest observed adverse effect level (LOAEL) for some of the patients may not have been as stringent as the criteria established for the PATS. In particular and among the mild transient reactions that did not meet the predetermined objective criteria, 13 additional patients experienced hives (a single hive in 8 cases, 2 hives in 4 cases, and 3 hives in 1 case, all lasting less than the stipulated 5 minutes). Had these 13 cases been counted as positive response to the single-dose challenge, the reaction rate would have

376 been 5.5%. Given these possibilities, the log-normal model used appears to be reasonable and

377 appropriately conservative for use in the estimation of EDs for peanut.

378

379 Population modelling of individual threshold doses can be used to establish public health measures

380 such as the control of precautionary allergen labelling (PAL). In Australia, a Reference Dose for

381 peanut of 0.2 mg peanut protein was established from estimates of the ED_{01} (2). The ED_{01} was

382 selected by the VITAL Scientific Expert Panel (VSEP) because it is predicted to protect 99% of the

383 peanut-allergic population. However, based on the mild and transient responses encountered in

384 PATS, the use of the ED_{05} as the basis for the peanut Reference Dose would be a more reasonable

385 and implementable risk management decision.

386

387 PAL abounds in many marketplaces but stakeholders find fault with the approach because use of

388 PAL bears little relationship to actual risk (13,14). Almost 50% of the study population were

389 routinely ignoring precautionary labelling. PATS has validated the $ED_{05,}$ so the medical and food

390 science communities, manufacturing industry, and public health authorities should consider

391 adopting this model. This would assist in establishing an ED_{05}-based peanut Reference Dose to be

392 used in quantitative risk assessment to underpin PAL, backed by sound scientific evidence, that

393 protects the vast majority of the peanut allergic community.

394

395 No centre appeared to have a uniquely more sensitive study population than the other two,

396 suggesting this protocol and the predetermined criteria used for assessing single dose OFC could be

397 used in other centres. Ireland had far more challenge-proven cases than the other centres but lower

398 average ages than the US centre, and Australian patients had larger peanut SPT and paid less

399 attention to precautionary advisory labels. These inter-centre demographic and diagnostic

400 differences did not influence the primary or secondary outcomes of the study.

401   The predetermined approach to offer the study to all peanut allergic subjects in 3 distinct

402   geographical regions, the comparison of characteristics of participants and non-participants, the

403   permissive entry criteria and the pre-determined conservative case definition combine to address

404   the most common criticism of OFC studies: How representative of the general peanut allergic

405   population are the subjects who volunteered? This study showed peanut allergic children in each

406   centre were broadly similar, that severe reactors were included and, critically, that participants

407   appeared not to differ clinically from non-participants. While we did not prospectively record

408   previous reaction severity, all subjects were recruited from referred populations seen for their

409   peanut allergy in tertiary/national referral centres, so it is likely the representation of the severe end

410   of the clinical spectrum of peanut allergy in this study population is at least similar than that

411   reported peanut allergy norms.

412

413   **Limitations of the study**

414   Many of the patients recruited were diagnosed without the gold standard double-blind placebo

415   controlled food challenge. However, the intended recruitment strategy was to recruit relatively

416   unselected but near-certain cases, to capture the whole spectrum of cases, which is often not

417   included in incremental dose challenge studies. Our data show no differences in demographic

418   details or serological findings between participant and non-participants or between reactors and

419   non-reactors or between the 8 most certain objective reactors and other groups. The inclusion and

420   exclusion criteria appear to have been well constructed, based on established clinical methods used

421   elsewhere, clinical history and SPT and spIgE levels above determined decision points (7).

422

423   Subjects did not undergo placebo challenges, just an active-dose cookie, given once. Placebo doses

424   would have required doubling attendances to more than 700 visits and we considered the projected

425   likelihood of significant reactivity of around 5% in the single dose study did not justify a placebo

426   arm. It is notable that 65% of subjects reported no reaction at all to the ED_{05} cookie, despite

427   knowing it was an "active" dose. Intentionally liberal documentation of reported symptoms and

428   having a set of fixed, pre-test criteria for an objective reaction allowed *post hoc* distinction of

429   subjective from objective reactors, though relatedness of any reaction to the single dose was

430   difficult in real time due to the lack of options normally available in routine OFC, such as waiting

431   longer between doses and repeating doses (1,7). Subjective reactors had lower pre-test FAQL than

432   objective reactors and non-reactors which suggests anxiety may play a role in reports of

433   mild/subjective reactions at low doses in the community and in DBPCFC (15) and also possibly in

434   reactions to placebo doses during DBPCFC (16).

435

436   PATS was an assessment of low-dose sensitivity in a population of peanut allergic subjects at a

437   single time point and further studies are needed to assess both population-level and individual

438   subjects' variation in low-dose sensitivity over time. Standard, incremental DBPCFC does not

439   correlate well with reported severity of community reactions (17) and dose is only one variable to

440   be considered in the difficult assessment of severity of food allergy. (18)

441

442   The PATS study offers a new clinical paradigm and methodology with regards to assessing clinical

443   risk; this current study may potentially define the 5% of patients who are most dose-sensitive. It

444   confirms previous findings that validated questionnaires assessing FAQL show patients gain nearly

445   as much from a "failed" OFC as they do from a "passed" OFC, probably due to decreased

446   uncertainty about the next and future reactions. (13). This tangible impact could promote adoption

447   of PATS single dose peanut challenges in units not currently performing diagnostic multi-dose

448   OFC.

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450 The single dose protocol does not replace current clinical food challenges which are critical for

451 definitive diagnosis of food allergy but would provide extra clinical information of patients' level

452 of risk, related to dose, and could help inform consumer choices and physician advice to patients

453 regarding PAL (14, 15); single dose challenges could be done before starting a progressive clinical

454 food challenge to identify the most highly sensitive patients and reduce any risks associated with

455 the use of higher doses used in clinical food challenges. PATS suggests clinical validation of other

456 allergenic food sources could be addressed in similar studies, where the population dose-

457 distribution has been modelled using sufficient threshold data. Clinicians may be able to use PATS

458 single dose OFCs widely as they are easier to perform than routine diagnostic OFC or DBPCFC.

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460 ***Conclusion***

461 The novel single dose OFC, based upon the statistical dose-distribution analysis of past challenge

462 trials, is a clinically safe and efficient approach to identify the most highly dose-sensitive

463 population of food-allergic people and it improves food allergy related quality of life. The

464 validation of the ED_{05} will also assist regulators, public health agencies and manufacturers in the

465 establishment of approaches to allergen management that will protect the vast majority of food-

466 allergic consumers/patients.

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Author's contributions

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476   JOBH is the guarantor of the study and wrote manuscript drafts.

477   JOBH, KA and SLT conceived the study initially.

478   WS and KA contributed to study design, clinical supervision and data analysis.

479   JN produced OFC materials, monitored the study and contributed to data analysis.

480   JB and ADG contributed to study design and data analysis.

481   GDG performed data analysis.

482   GZ and LG contributed to study design, selection of statistical methods and challenge performance.

483   All authors have contributed to manuscript drafts and have seen and approved the final version

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489

Conflicts of Interest

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491   JOBH, KJA, LCG, GZ, JN, GDG, ADG none to declare. WGS receives consulting fees from

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494   Resource Program consortium and from the U.S. Department of Agriculture. SLT and JB receive

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496   immunoassay kits. SLT receives consulting fees from ConAgra Foods (Omaha NE, USA),

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1   **Legends**

2   Figure Flow diagram of subject recruitment and participation

3   Table 1. Inclusion and Exclusion Criteria

4   Table 2. Demographic comparison of participants to non-participants

5   Table 3. Primary Outcomes (reaction to single dose food challenge) per centre.

6   Table 4. Participants who met the predetermined objective reactivity criteria/case definition

7   Table 5. Reaction type vs. Mean values for Skin and Blood Tests

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29   **Table 1. Inclusion and Exclusion Criteria**

Inclusion Criteria	Exclusion Criteria
Age between one to eighteen years old inclusively.	Medically unfit for challenge according to local unit OFC guidelines/protocol (e.g. high fever, unwell with intercurrent illness)
Evidence of peanut allergy by one of the following;	Oral corticosteroids within 14 days prior to challenge
History of unequivocal exposure (including accidental) and typical acute allergic reaction within the preceding 2 years and positive peanut SPT (performed according to local clinical protocols) /specific IgE.	Episode of anaphylaxis of any cause in the 4 weeks prior to challenge
Positive oral food challenge with peanut performed within 2 years - either open oral food challenge or DBPCFC (Double-blind, placebo-controlled food challenge)	Use of antihistamines within 5 days of oral food challenge
Peanut never ingested, but sensitisation to peanut above the 95% positive predictive value (PPV) for clinical allergy, i.e. peanut serum IgE $\geq$ to 15kU/L (by CAP FEIA) and/or peanut SPT wheal size $\geq$ to 8mm (7) within 2 months of the single dose challenge.	Asthma that is not well controlled as demonstrated by FEV_1 <85% of predicted best.

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43   **Table 2. Demographic comparison of participants to non-participants**

	Participants			Non-Participants		
	Cork	**Melbourne**	**Boston**	**Cork**	**Melbourne**	**Boston**
Initial Number	124	126	128	63	24	53
Sex (%Male)	61%	56.3%	55.5%	60.3%	70.8%	71.7%
Age (Mean yrs)	6.36	7.63	6.55	6.78	8.54	6.65
Final Number*	124	126	128	63	24	35
Inclusion criterion met:**						
Typical reaction<2years	68	60	74	38	12	19
Positive OFC<2years	43	16	2	8	1	2
SPT/spIgE > 95% PPVs	13	50	52	17	11	14

44   *18 participants in Boston did not wish to participate immediately after initial recruitment and therefore no
45   diagnostic information was gathered.
46   ** Many subjects met both entry criteria 1 and 2 but only the single one entered in the restricted data file option
47   is reported here.
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68   **Table 3 Primary Outcome (reaction to single dose food challenge) per centre**

	Total	Cork	Melbourne	Boston
Participants				
Active Eligible Participants (completed OFC)	378	124	126	128
Outcome Group				
Total	378	124	126	128
Non-reactors	245	94	65	86
Reactors	133	30	61	42
Subjective Reactors	67	19	30	18
Objective Reactors				
Total Objective	66	11	31	24
Meeting predetermined criteria	8	1	3	4

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91   **Table 4. Details of 8 subjects who met the predetermined objective reactivity criteria/case definition**

Participant Number	Location	Age (yrs)	Sex	Diagnostic method	Peanut Wheal (mm)	Peanut SpIgE kUA/L	SpIgE rArah1	SpIgE Arah2	Outcome
35	Ireland	11	Female	History of typical exposure & reaction & positive SPT/ spIgE	15	69.10	11.20	59.20	Rhinoconjunctivitis
40	Australia	15	Male	History of typical exposure & reaction & positive SPT/ spIgE	13	2.06	0.53	1.74	Urticaria
43	Australia	9	Male	History of typical exposure & reaction & positive SPT/ spIgE	18	N/A	N/A	N/A	Vomiting
95	Australia	2	Female	Peanut never ingested but positive SPT/spIgE> 95% PPVs	13	N/A	N/A	N/A	Vomiting
31	U.S.	9	Male	Peanut never ingested but positive SPT/spIgE> 95% PPVs	11	0.36	0.10	0.14	Urticaria
97	U.S.	2	Male	History of typical exposure & reaction & positive SPT/ spIgE	N/A	100.00	14.80	100.00	Urticaria
109	U.S.	1	Male	History of typical exposure &reaction & positive SPT/ spIgE	N/A	57.70	0.10	49.60	Urticaria
124	U.S.	4	Male	History of typical exposure & reaction and positive SPT/ spIgE	N/A	46.70	14.70	16.20	Rhinorrhoea

92   **Table 5. Reaction type vs. Mean values for Skin and Blood Tests**

	Total IgE	Peanut spIgE	Peanut SPT Wheal (mm)	rAra h1 spIgE	rAra h2 spIgE	rAra h3 spIgE	rAra h8 spIgE	rAra h9 spIgE
<u>Type of reaction (n)</u>								
Non-reactor (245)	490.46	28.18	11.69	11.11	22.52	4.88	1.49	0.74
Subjective (67)	1164.89	46.07	15.23	23.42	32.86	9.33	0.74	0.11
Objective (66)	1130.80	39.46	13.60	14.87	31.90	3.13	1.21	0.19
Satisfies pre-determined criteria (8)	290.67	45.99	14.00	8.18	45.03	2.35	0.13	0.31

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Figure X. Flow of participants through study

Assessed for eligibility (n =518)

Excluded by clinician (n= 21)

Reason given by clinician:
➤ Lost to follow up (n=15).
➤ Did not met inclusion criteria (n=6).

Withdrawn by parent/child (n =116)

No reason given for withdrawal (n=18)

Reason given by parent:
➤ Too nervous/worried about serious reaction (n=29).
➤ Too busy/no time to participate (n=29).
➤ Live too far away from study centre (n=10).
➤ Wishes to continue to ignore/adhere labelling (n=8).
➤ Child does not want to participate (n=8).
➤ Wishes to continue to ignore/adhere labelling & are worried about potential reaction (n=7).

Allocation of participants based on eligibility and consent (n=518).

Total number of participants _not_ eligible/withdrawn (n=137)

Total number of participants eligible and provided consent (n=381)

Excluded at start/during protocol by clinician (n=3)
Reason given by clinician:
➤ Child too unwell to start protocol (n =1)
➤ Incomplete ingestion of peanut cookie (n=2)

Total number of _non-_participants at end of protocol (n =140)

Total number of participants at end of protocol (n =378)

Total number of non-reactions (n=245)

Total number of reactions (n=133)

Total number of subjective reactions (n=67)

Total number of objective reactions (n=66).

Total number that met pre-determined objective criteria (n=8).