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This is a repository copy of *UK NEQAS survey of allergen component testing across the United Kingdom and other European countries*.

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Title: UK NEQAS Survey of Allergen Component Testing across the UK and Europe

Short title: Allergy diagnostics survey

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Molecular-based allergy, component resolved diagnostics, allergenic components, allergic risk stratification, allergy testing

Abbreviations used:

MA – molecular-based allergy

CRD – component resolved diagnostics

LTP – lipid transfer protein

UK NEQAS – UK National External Quality Assurance Service

Summary

The clinical utility of molecular diagnostic approaches in allergy investigation is increasingly being recognized to play a significant role in the management of allergic patients. Determining the sensitisation pattern, which is best achieved through the use of component resolved diagnostics (CRD), allows effective risk stratification, appropriate treatment and patient selection for immunotherapy. In order to assess the diagnostic service provisions for *in vitro* allergy testing across Europe, a survey was carried out via the total IgE and Specific IgE external quality assurance schemes run by UK NEQAS Immunology, Immunochemistry & Allergy.

This survey assessed allergy testing and in particular allergen-components offered by the laboratories and found a wide variability in service provision, particularly between the UK and EU. Furthermore, there was lack of standardisation for acquisition of clinical information to aid allergen (and component) selection, gating strategy, testing algorithms and clinical interpretation. Interestingly, a significant proportion of laboratories (the majority from EU) stated that they 'used' the results for peanut components for risk stratification. However, vast majority of participants were unaware of guidelines relating to the use of allergen component testing and agreed further education would assist in reaching a common platform.

Hence, this survey has highlighted that although CRD has been adopted into routine diagnostics across Europe; it is potentially compromised by lack of standardised protocols and guidance sources. Consequently, there is a need for local or national standards and education through External Quality Assurance services on the performance and application of CRD into allergy investigation.

Max Word count = 250 (above summary 248)

Introduction

Recent developments in molecular techniques have given rise to advances in the knowledge of properties of specific allergens, and has aided their clinical utility in both diagnosis and management of allergic patients. These advances have allowed for the use of specific allergen components in *in vitro* diagnosis in what is termed Molecular diagnosis, or Component Resolved Diagnosis (i.e. identifying specific IgE to distinct allergenic sub-components of the whole allergen extract). Component Resolved Diagnosis (CRD) provides clinicians with an extended diagnostic toolkit with potential for cross-sensitisation profiling, risk stratification, and allergen identification for improved patient management [1, 2]. Benefits to the patient may include negating the need to undergo the risk of an Oral Food Challenge where sensitisation to high-risk components is present, or eliminating the requirement for dietary exclusions where cross-reactive components associated with low risk of systemic reactions are identified [3]. These potential patient benefits are important when considering patient health, quality of life, and the risks and costs associated with challenge tests.

There is worldwide data suggesting that the prevalence of allergy is increasing. A recent systematic review of food allergy across Europe assessed prevalence of food allergy in both adults and children [4]. A pooled lifetime prevalence of

self-reported allergy was found to be 17.3%, with point prevalence of 5.9%. Sensitisation to more than one food, as indicated by specific IgE, was found in 10.1%. However the prevalence of true allergy, as confirmed by food challenge (either open or double blind placebo controlled), was much lower at 0.89% in children and 0.99% in adults (overall point prevalence of 0.93%). This distinct discrepancy between self-reporting, *in vitro* sensitisation assessment and confirmed food allergy illustrates the need for a greater use of more accurate diagnostic testing to diagnose or exclude allergy. The difference in prevalence between self-reported allergy and clinically confirmed allergy was reflected across Europe [4, 5], with the greatest difference seen in Northern Europe (14.51% self-reported; 1.12% confirmed point prevalence). Unfortunately, clinical false-positivity, the prevalence of detectable but irrelevant sensitisation to whole allergen extracts which does not lead to clinical symptoms is often much higher than true clinical allergy for many allergens.

When assessing and reporting allergy; the distinction between asymptomatic sensitisation, irrelevant in-vitro cross-reactivity and a clinically symptomatic allergy is vital. CRD have been found to have a useful role in distinguishing allergy due to primary allergen sensitisation from benign cross-sensitisation due to structural similarities between allergens, but which results in positive testing. Panels of CRD allergens have shown to be of clinical value in

prevalence studies, and sensitisation patterns can sometimes indicate likely severity of symptoms (e.g. Ara h 2 as a risk factor for positive challenge testing) or mostly benign cross sensitisation (e.g. Cross reactive carbohydrate determinants or labile PR10 proteins). Peach and apple are known to be frequent sensitisers in Europe [6]. Sensitisation LTPSs (Lipid Trasfer proteins) such as apple Mal d 3 and Peach Pru p 3 are linked to clinical reactions [3].

The European prevalence of peanut sensitisation may be as high as 2.7% [5] but only 1/5 of these may have clinically significant allergic symptoms. In a study of childhood peanut allergy, 22.4% of sensitised 8 year olds (of 933 participants) had confirmed peanut allergy by double blind placebo controlled food challenge [7]. Comparison of sensitisation rates to individual components determined the peanut component Ara h 2 to be the best predictor of clinical outcome. The clinical utility of Ara h 2 was also shown by a prospective study comparing specific IgE to peanut Ara h 2 and outcome of food challenge [8]. Ara h 2 and Cor a 14 were better discriminators of allergy from tolerance than whole peanut or hazelnut extract respectively. In a separate study, Ara h 2 sIgE had the best correlation with challenge outcome, superior to Ara h 1, 3, 8, 9 and peanut specific IgE [9]. The close association of an immunodominant major component sensitisation (often referred to a species specific sensitisation) to probability of clinical allergy is to be expected; components

are usually named in order of discovery or because they are ranked in order of frequency of sensitisation in a population and this mirrors the order of immunodominant allergens in a response. When cross reactive sensitisation is present this obscures the presence or absence of species-specific sensitisation, thus identifying or eliminating signal from cross-reactive components **is a key feature in component assay performance**. Thus Ara h 1, 2, 3 and 6 are also species-specific allergen components associated with clinical peanut allergy, but Ara H2 is most strongly associated and the best predictor in isolation. Conversely, sensitisation to the cross-reactive PR10 protein Ara h 8 alone is often a marker of false positivity due to pollen sensitisation and associated with minor reactions predominantly.

The use of component testing is increasingly used in the clinical management of patients reducing the need for risky food challenges for confirmation of allergy, allowing effective risk-assessment of patients without challenge, and accurately identifies sensitising allergens for appropriate management including patient selection for immunotherapy. It may be helpful that laboratories supporting allergy clinics provide these services as part of their testing repertoire. In order to assess service provision, UK NEQAS Immunology, Immunochemistry & Allergy (IIA, Sheffield, UK) conducted a survey of participants.

Methods

Allergen component testing survey was distributed via UK NEQAS IIA as part of the total IgE scheme (n=248) and specific IgE scheme (n=383) to the participating laboratories offering allergy diagnostic testing to ascertain the breadth of allergy services and local practices including allergen component testing.

The survey contained 25 questions; eight questions focused on the geographical location of the participating laboratory, its workload, requesting pattern and the basic diagnostic allergy services provided, whilst the remaining seventeen focused particularly on the use of allergen component testing.

The responses from this survey were collated in a spreadsheet for analysis.

Results

Overall 19% (n=73) of all participants in the specific IgE scheme surveyed (n=383), provided responses. However, not all answered every question and therefore the response rate is quoted for each.

Location, allergy workload and requesting sources

In terms of the geographical location, over half (n=41, 56%) of the responding laboratories were located in the European Union (EU) whilst 26% (n=19) were from the UK and a smaller number (n=13, 18%) were outside the EU.

In 2013 and 2014, it appears that on average 40% of respondents performed up to 10,000 allergy tests per annum. Roughly 20% of responding laboratories performed between 20,000 to 30,000 tests (**Table 1**). Interestingly, a small number of laboratories (n=15 in 2013 and 21 in 2014) performed the highest number of tests; between 30,000 and 100,000+ tests per annum (**Table 1**).

There was a wide variation in the number of allergy test requests coming from both primary and secondary care settings. Of 73 respondents, 32% (n=23) stated that up to 20% were from a source other than primary or secondary care (**Table 2**). This may reflect private allergy testing provided in some areas.

Receipt and processing of allergy requests

More than half of the respondents (55% n=35 out of 64 total) vetted allergy requests on receipt for appropriateness. The majority of these were EU (n=17) laboratories followed by the UK (n=10).

Interestingly, most services do not require a completed allergy questionnaire proforma to provide clinical information for interpretation and allergen

selection: the majority 95% (n=62) of total 65 respondents answered ‘no’; whilst only 5% (n=3) did so, (2 from UK and 1 EU laboratory).

Out of 67 respondents, the majority (65% n=44) performed allergy tests on all allergens requested, whilst 35% (n=23) did not. These may be due to processing issues e.g. insufficient sample received, or vetting protocol upon allergy request receipt to modify requests to ensure relevant testing.

In total, 23% (15/64 respondents) used both allergen mixtures and panels whilst a higher proportion, 45% (29/64 respondents), used allergen mixtures only. Phadiatop methodology was used by 9% (n=6) of the responding 64 laboratories.

Allergen component testing

Availability

Allergen component testing was routinely offered by 78% (n=45) of the 58 respondents. This included 26 EU, 11 UK, and 8 Non-EU laboratories.

Access to Primary care

Furthermore, a significant proportion (74% n=41) of the responding 56 laboratories (mainly located in EU n=25), permitted allergen component requests from GPs and/or primary care health professionals.

Gating policy

Interestingly, 35% (n=19/54 respondents) stated that component testing is only performed if the allergen screen was positive. Therefore, the majority (65% n=35) appear to perform the testing regardless of the allergen screen outcome. There were roughly an equal proportion of laboratories stating that component testing was allergen dependent (yes, 53% n=26) and allergen independent (no, 47% n=24).

Test selection policy

When asked if there was a testing algorithm for allergy and / or allergen components testing, a significant proportion of the 55 respondents did not have any algorithm for allergy (n=34, 63%) or for the components (n=38, 67%). However, a small group (n=21 and n=18 respectively) of laboratories stated they had algorithms for allergy and component testing respectively. These were mainly located in EU countries outside the UK (n=10/9 respectively) **figure 1**.

Methodology

The predominant method used for allergen component testing was unsurprisingly that of the largest test provider (Phadia ImmunoCAP) for 82% (n= 42) of 51 respondents. Hence the measuring units were reported in the majority

as KU/L (n=20) and KUA/L (n=18). Four laboratories used the semi-quantitative ISAC (Immuno Solid-phase Allergen Chip) alone. Two laboratories stated that they perform both single component and ISAC testing.

Cut-off levels

The vast majority of the 45 responding laboratories reported the cut-off range for allergen components to be ≤0.35 KUA/L (47% n=21) followed by ≤0.1 KUA/L (33% n=15) whilst a small proportion reported other variations of uncertain provenance such as 0.3ISU-E, 1.5 AU/mL, >0.35 or even <0.01KUA/L. The survey did not explore if any such alterative were locally validated according to ISO requirements.

Samples

All 47 respondents (100%) stated 'serum' as the preferred matrix including two EU laboratories that accept both serum and plasma. Of the 42 respondents, 57% (n=24) stated the minimum volume for testing to be 0.3ml, followed by 0.5ml in 33% (n=14) and 1.0ml in 10% (n=4) of the responding laboratories.

Repertoire

All laboratories were asked if they offered testing for 17 common allergens. The results are summarised in **figure 2**. Approximately two thirds of the respondents provided either 'yes/no' response. The majority offer components

for: a) peanut [n=46], b) egg [n=42], c) venoms [n=37], followed by nuts [n=36] and Omega-5-gliadin [n=36]. On average, more laboratories do provide components for all of the listed allergen categories except one (meat alpha-gal) as illustrated in **figure 2**.

Use of recombinants in peanut allergic patients

Routine diagnosis

47 laboratories responded to whether they routinely performed allergen component testing for peanut positive patients. Only 38% (n=18) answered 'yes' including 12 EU laboratories, while the remaining 62% (n=29) answered 'no'.

Risk stratification

Interestingly, a significant proportion, 74% (n=34) of 46 respondents stated that they 'used' the results for peanut components to stratify clinical risk of patients having a significant reaction. This included 21 EU, 8 UK and 5 Non-EU laboratories.

The participants were asked if they felt there was sufficient understanding regarding allergen component testing amongst: a) Immunology laboratory staff,

b) Hospital specialists and c) General Practitioners. The results are summarised in **figure 3**. A key finding was that 78% (n=40) of respondents agreed 'yes' for immunology laboratory staff, while 92% (n=47) agreed 'no' for general practitioners. There appeared to be a similar perception of need for enhanced understanding amongst hospital specialists, where a somewhat similar 42% (n=22) were felt to have sufficient understanding and 58% required educational input.

Guidance

Finally, in order to gauge participants' awareness of any national guidelines relating to allergen component testing, it transpired that the vast majority (80% n=41 out of 51 respondents) were not aware of any such guidance material. Those answered 'yes' (n=10) specified various sources including BSACI, WAO consensus document, NICE, EAACI and local clinical steering group approach.

Need for an Allergen component EQA Scheme

84% (n=43 out of 51 respondents) of respondents would be interested in participating in a pilot EQA scheme for allergen component testing.

Discussion

This survey provides an overview of the provision of laboratory diagnostic services and use of allergen component testing across the UK and Europe.

Overall, it confirms there is a wide variation in laboratory practices even within the same geographical location.

In terms of laboratory testing, it appears that a minority of laboratories offer more comprehensive testing including the allergen components, whereas others only provide the basic specific IgE screening tests.

The receipt and processing of samples is also highly variable, in that only a minority of laboratories appear to have protocols in place to demand-manage their workload or ensure appropriate test selection, such as vetting of allergy test requests and demand management appeared to be most common in non-UK laboratories.

Only a minority (n=3) restrict processing of allergy requests to those that have an accompanying completed questionnaire proforma (to ensure that sufficient information is available to ensure appropriate testing and useful interpretation). The vast majority of laboratories perform testing for all allergens requested by the clinician, including mixtures and panels and therefore are totally dependent on the clinician's knowledge to ensure appropriate test selection and interpretation

NICE Guidance (DG24) in the UK has recently been published which recommends that only experienced specialists should utilise multi-parameter

component testing chips like the ISAC, noting that their interpretation is complex and the evidence base currently insufficient to make further recommendations [10]. Worldwide, a higher proportion of laboratories permit component testing requests from primary care professionals (GPs), who may or may not have the knowledge and expertise to interpret the results accordingly (**figure 3**). Despite this the laboratories generally reported that they felt knowledge of allergen component testing was suboptimal in a large proportion of requesters from both primary and secondary care.

A few laboratories restricted availability of allergen component testing to primary care and reserved it exclusively for immunology consultants, allergy specialists, and paediatric allergy clinicians. This approach is justified by the need for careful clinical history taking skills and clinical judgement in selecting and interpreting tests[10].

The existing repertoire of components for common foods was widely available. Most respondents provided of tests for various allergen component categories including peanut, egg, venom, and nuts (**figure 2**). In addition, some of the non-UK EU laboratories provided component testing for additional allergens such as wheat, fruits, animals, etc. However, fewer laboratories (38% n=17 of 45 respondents) reported the availability of very specific components for rare

allergies e.g. meat alpha-gal component testing for delayed-type anaphylaxis to red meat and chimeric anti-cancer drug 'cetuximab' [1].

Harmonisation of diagnostic approach through agreed algorithms appears to be lacking. Although allergen component testing is offered routinely by a significant number of laboratories (78% n=45 of 58 respondents), algorithms for selecting or interpreting allergen components or allergy testing in general are rarely used, and many laboratories do tests as requested and do not modify or gate the requests. Consequently, the majority of laboratories reported performing these tests regardless of the allergen screen outcome. Interpreting the result of a positive component test where the whole extract screen is negative will be a challenge, and some might argue that it is a waste of resource to do specific testing on screen-negative samples.

Furthermore, there was little awareness of national guidelines relating to component testing amongst the users (80% n = 41 out of 51 respondents lacked awareness). A significant proportion of users (74% n=34 of 46 respondents) reported the local 'use' of peanut components to stratify clinical risk of patients in peanut challenge. However, only 38% reported performing routine allergen component testing for peanut positive patients at the time of first testing. This in itself may indicate variability in practice.

Conclusion

This survey highlights the increasing use of CRD, accompanied by apparent lack of harmonisation of approach and identifies concerns about the need for education of test requesters in primary and secondary care settings. It also demonstrates geographical differences in terms of testing across the UK and the rest of Europe.

Agreed local or national guideline may help to harmonise laboratory diagnostic strategies and illustrates the need for External Quality assessment of the test performance of CRD, together with enhanced education on their use.

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Conflict of Interest

None disclosed by all authors.

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Tables and Figures

Table 1: No of allergy tests performed per annum in the year 2013 and 2014

No of allergy tests per annum	No of participants	
	2013	**2014**
0-10,000	29 (41%)	28 (38%)
10,000 – 20,000	11 (15%)	10 (14%)
20,000 – 30,000	16 (23%)	14 (19%)
30,000 – 50,000	5 (7%)	8 (11%)
50,000 – 100,000	5 (7%)	7 (10%)
100,000+	5 (7%)	6 (8%)
Total respondents	**n = 71**	**n = 73**

Table 2: Breakdown of allergy testing requests from various sources

% of allergy tests	**Requesting Source Breakdown**			
	Primary care	**Secondary care**	**Other**	
0 – 20% tests	23 (32%)	12 (17%)	23 (70%*)	
21 – 40% tests	16 (22%)	12 (17%)	7 (21%*)	
41-60% tests	13 (18%)	22 (31%)	2 (6%*)	

61-80% tests	9 (13%)	12 (17%)	0	
81-100% tests	11 (15%)	12 (17%)	1(3%*)	
Total respondents	**72**	**70**	**33**	

***** Percentage based on total number of respondents (33) for this category only instead of all (73) respondents.

Figure 1: Breakdown of responses to question 13 - whether participants have algorithms for allergy and/or allergen component testing?

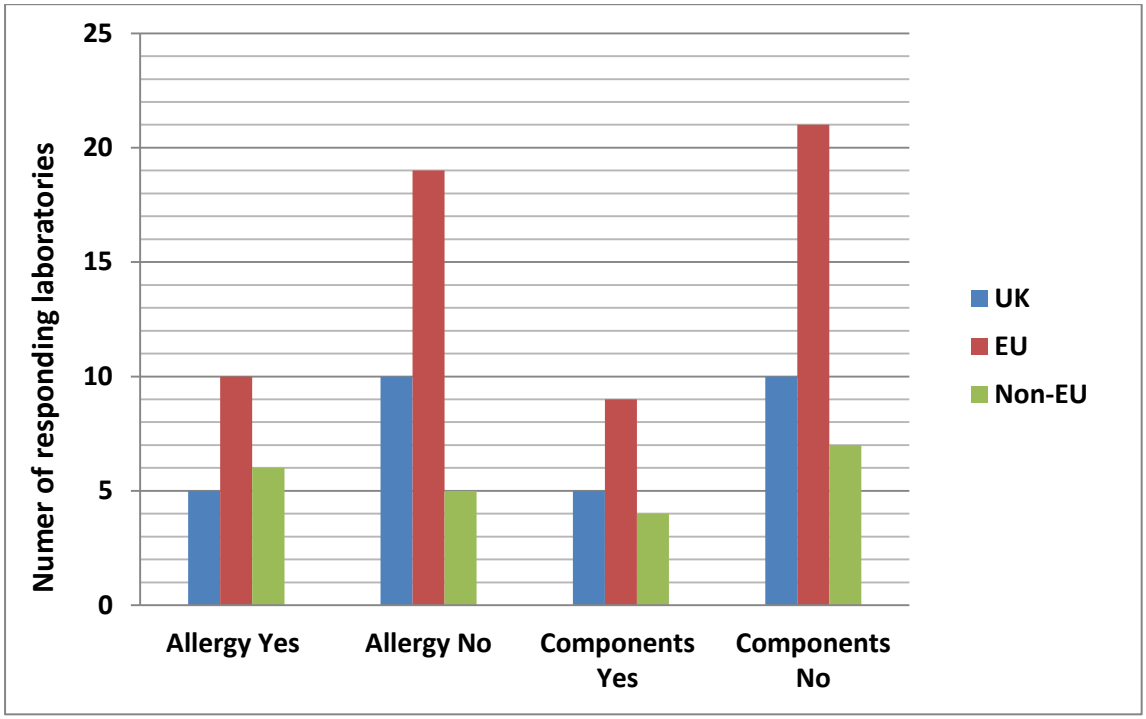
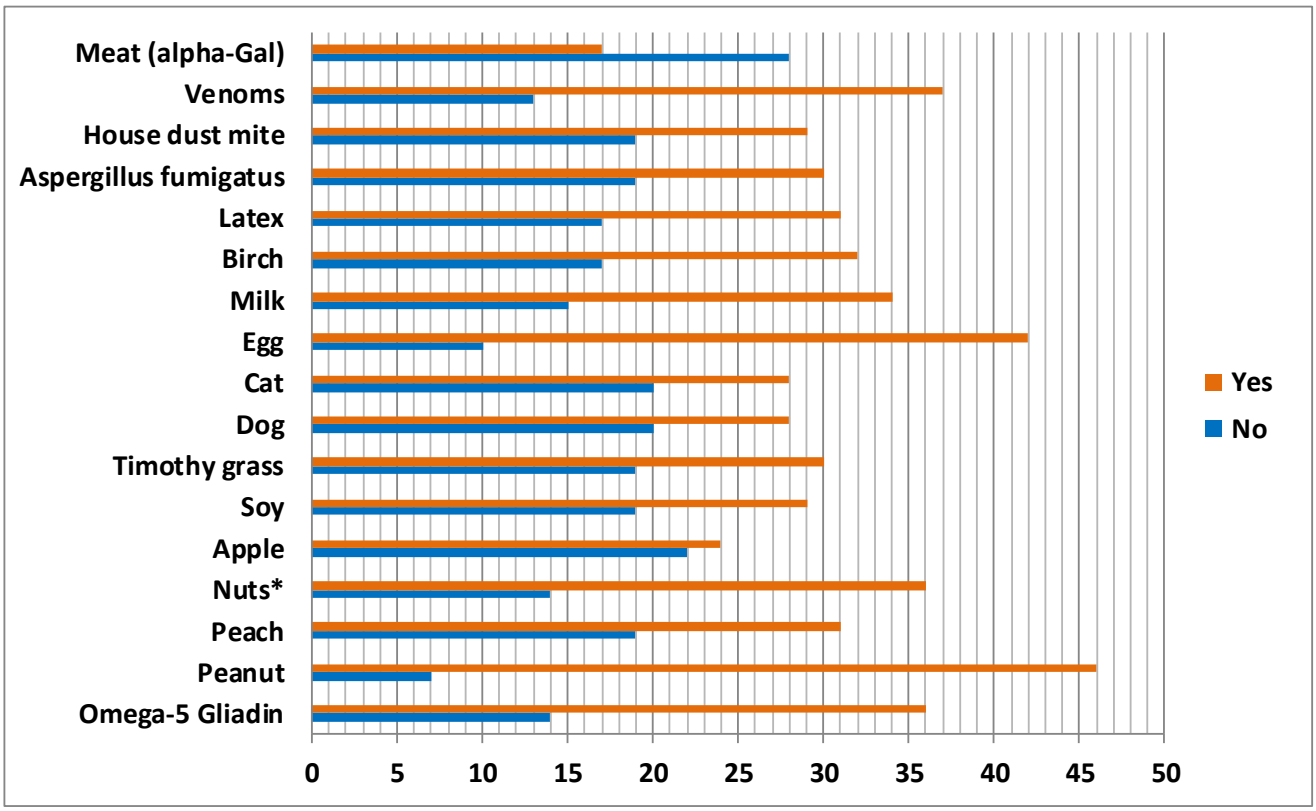


Figure 2: Results of question 15 - which allergen components are offered?



Nuts* - included Brazil/Hazelnut/Cashew/Walnut

Figure 3: Results of question 22- Do you feel there is sufficient understanding regarding allergen component testing amongst: a) Immunology laboratory staff, b) Hospital specialists, and c) General Practitioners?