i. **Conflicts of interest:** LS, JE, NA, LL, SV and MS have no competing interests to declare.

ii. Financial support: no financial support

iii. Keywords: Multiplex allergy array, molecular allergy diagnostics, ALEX, ISAC, pediatric allergy

iv. Main text

To the editor,

Recently a new multiplex array in molecular allergy diagnostics, ALEX (Allergy Explorer, Macro Array Diagnostics, Vienna, Austria), was launched and became commercially available in 2019. This array offers more than 120 allergen extracts and 170 molecular components (ALEX^2) of inhalation, food, animals, latex and insect allergens. The ISAC (Immuno Solid-phase Allergen Chip, Thermo Fisher Scientific, Uppsala, Sweden, available since 2008) is the most frequently used and studied multiplex array to date, offering 112 different molecular components. Molecular allergy diagnostics is based on detection of specific IgE (sIgE) for allergen components and applied in patients with multiple allergies or unexplained (anaphylactic) reactions to find a causative allergen. Analyzing molecular components is considered a third-level diagnostic tool, after Skin Prick Test (SPT) and sIgE for whole allergens extracts. It helps clinicians to distinguish between clinically relevant (e.g. storage proteins (SP)) and less relevant sensitizations (e.g. cross reacting PR-10 proteins (PR10)). ALEX reported their scores as kUA/L, the same unit used for ImmunoCAP (Thermo Fisher Scientific), classified in five different categories. ISAC results are reported as semi-quantitative ISU units classified in four different categories. In addition, a different range is used (0.3–50 kUA/L for ALEX compared to 0.3-200 ISU-E for ISAC). This is the first study comparing ALEX and ISAC diagnostic performance in children with multiple allergies. Previous studies compared both tests for inhalation allergens in adults [1,2] or for inhalation and food allergens but without specific results for the included children[3]. In our study, ALEX test was conducted in ten children with multiple allergies using same stored serum samples in which ISAC test was previously performed in 2020.

Results

Ten patients with multiple food allergies were included with a mean age of 11,8 years (range 7-16). The majority was male (80%) and 80% was diagnosed with eczema, 70% with asthma and 90% with allergic rhinitis, all requiring medication (Table 1).

Overall agreement

The overall agreement between ALEX and ISAC was good with 86,2% agreement (both positive / both negative) for all shared allergen components between ALEX and ISAC (n=102) with a negative percent agreement (NPA, both negative) of 90,3% and a positive percent agreement (PPA, both positive) of 79,5% (Figure 1). Overall agreement between ALEX and ISAC based on four sensitization categories (<0.3 (0) (negative), 0.3-1 (1) (low), 1-15 (2+3) (moderate-high), >15 (4) (very high)) was 79,4% (Figure 2).

Focus on food allergens

For egg, cow's milk and peanut ALEX offers three additional components (Gald4, Bosd2 and Arah15) and three extra whole allergen extracts (egg yolk, egg white and cow's milk). For nuts, ALEX provides five extra whole allergen extracts (cashew, brazil, pecan, macadamia and almond) and eight extra allergen components for hazelnut, walnut, macadamia and pistachio (Cora11; Jugr2, Jugr4, Jugr6; Mac I 2S Albumin; Pisv1, Pisv2 and Pisv3). For legumes, ALEX offers whole allergen extracts of peas, chick peas, lentils and green bean and Glym8 (storage protein) soy component. For cereals, ALEX contains whole allergen extract and/or components for wheat, oats, quinoa, buckwheat, barley, millet, lupine, rye, spelt, rice and corn. In evaluating fish and seafood ALEX provides whole allergen extracts and extra components for eight fish species (codfish, carp, herring, mackerel, salmon, stingray, swordfish and tuna), three prawn species (north sea shrimp, pacific white shrimp and northern pink shrimp), crab and lobster and five mollusks species (clam, mussel, oyster, scallop and squid).

Egg, cow's milk and peanut

Our study included two children with egg allergy, three children with cow's milk allergy and four patients with peanut allergy (Table 1). We found comparable results for most relevant overlapping components in evaluating cow's milk, egg and peanut allergy.

Nuts

All patients in this study were allergic to (certain) nuts (Table 1). Between overlapping nut components (hazelnut, walnut, cashew- and brazil nut) the overall agreement (both positive / both negative) was 83% (with a NPA of 88% and PPA of 79%). We found two major differences in sensitization for storage proteins of hazelnut (Cora14) and walnut (Jugr1) with unknown clinical relevance in two patients (no challenge tests performed). Verification of this difference was done by performing a monoplex sIgE test (ImmunoCAP) on the same serum sample. In patient 2 (Cora14) sensitization conform ISAC and in patient 5 (Jugr1) sensitization conform ALEX was confirmed to be right.

Legumes

Comparable results were found for overlapping soy components, except a clinically irrelevant difference in patient 1 who tolerates small amounts of soy. In this patient, Glym5 was negative in ALEX while moderately elevated in ISAC. Furthermore, ALEX revealed sensitizations for chickpeas and lentils with unknown clinical relevance in three patients.

Cereals

Between overlapping wheat components comparable results were found. In patient 2, results were different for buckwheat component (storage protein, Fage2), with no sensitization in ISAC while moderate sensitization in ALEX together with high sensitization for whole allergen extract. Because of unexplained anaphylaxis a buckwheat challenge test was performed, however this was negative. In addition, in seven patients ALEX revealed sensitizations for buckwheat with unknown relevance in five of them.

Fish/seafood

Comparable results were found for overlapping components (codfish and black tiger prawn). However, in patient 5 and 8 with fish allergy ALEX showed no sensitization for tuna whole allergen extract while sensitization for the parvalbumin component (Thua1) was very high. In all other fishes sensitizations for parvalbumin components were considerably higher than whole allergen fish extracts.

Other remarkable findings

Many extra sensitizations were found by ALEX all with unknown clinical relevance for a.o. fenugreek (n=4), poppy seeds (n=8), lupin (n=5), beer yeast (n=3), storage mites including Acarus siro (n=7), honeybee (n=1), and cricket, mealworm and grasshopper (n=5). Patient 8 with dust mite and seafood allergy (sensitized for tropomyosin) showed very high sensitizations for cricket, mealworm and grasshopper and based on this result he was advised to avoid those insects in his diet. A challenge test with poppy seeds was performed in patient 2 due to unexplained anaphylaxis and high sensitization which was negative.

Conclusion

This is the first study comparing ISAC and ALEX results in atopic children. Good agreement was found for shared egg, cow's milk, peanut, codfish/prawn, soy and wheat components. In evaluating nut allergy some differences were found. ALEX provides more sensitization results (including whole allergen extracts besides components) for nuts, cereals, seeds, legumes, fish and seafood which can help clinicians to optimize and personalize dietary advices. In addition, ALEX provides more information regarding fruits, vegetables, meats, spices and insect venoms. However, more results on sensitization with unknown clinical relevance could potentially increase clinical complexity, resulting in an increase in further testing (a.o. challenge tests). This happened in one patient with unexplained anaphylaxis where both poppy seeds and buckwheat challenge tests were performed with negative results. Furthermore, ALEX and ISAC use different units (kU/L vs ISU-E) and ranges so clinicians will need some experience in translating it into their daily clinical practice. Because of the small sample size, analysis was limited to a descriptive case study. More data is needed to unravel sensitization patterns and clinical relevance in severe atopic children. ALEX seems to be an interesting new diagnostic tool in molecular allergy diagnostics to support pediatric allergists in precision medicine for severe atopic children with multiple allergies.

Laura JH Sonneveld, MD

Joyce AM Emons, MD PhD

Nicolette JT Arends, MD PhD

Lonneke J Landzaat, MD

Department of Pediatric Allergy, Sophia Children Hospital, Erasmus MC University Medical Centre, Rotterdam, The Netherlands

Sharon Veenbergen, PhD

Marco WJ Schreurs, PhD

Department of Immunology, Laboratory Medical Immunology, Erasmus MC University Medical Centre,

Rotterdam, The Netherlands

v. References

1. Heffler E, Puggioni F, Peveri S, Montagni M, Canonica GW, Melioli G. Extended IgE profile based on an allergen macroarray: a novel tool for precision medicine in allergy diagnosis. *World Allergy Organ J.* 2018; 11:1-7
2. Bojcukova J, Vlas T, Forstenlechner P, Panzner P. Comparison of two multiplex arrays in the diagnostics of allergy. *Clin Transl Allergy*. 2019; 9:31.
3. Scala E, Caprini E, Albeni D., Meneguzzi G, Buzzulini F, Cecchi L, Villalta D, Asero R. A qualitative and quantitative comparison of IgE antibody profiles with two multiplex platforms for component-resolved diagnostics in allergic patients. *Clin Exp Allergy.* 2021;00:1-10.

vi. Table legends

Table 1 Patient characteristics

vii. Figure legends

Figure 1 Scatter plot of all ALEX and ISAC sIgE allergen overlapping components test results.

Figure 2 Agreement of ALEX versus ISAC test results by classes defined for ISAC in 10 patients

= patient number. *Note:* ALEX results are classified in five different categories: negative (<0.3 kUA/L), low (0.3-1 kUA/L), moderate (1-5 kUA/L), high (5-15 kUA/L) and very high (>15 kUA/L). ISAC results are classified in four different categories: negative (<0.3 ISU-E), low (0.3 – 1.0 ISU-E), moderate-high (1-15 ISU-E) and very high >15 ISU-E). For overall class agreement between ALEX and ISAC results were classified in four sensitization categories (<0.3 (0) (negative), 0.3-1 (1) (low), 1-15 (2+3) (moderate-high), >15 (4) (very high)).