LABORATORI ANALISI AMODIO

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Allergy Explorer

diagnostica molecolare

(informativa medico/paziente)

ALEX

ALLERGY EXPLORER

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Allergia: il percorso diagnostico

“I **test di primo livello** in ambito allergologico sono i **test cutanei**. Questi però, in alcuni casi, non sono in grado di identificare quale sia l’allergene principale responsabile della reazione allergica; ciò si verifica per via del fenomeno della **multi-sensibilizzazione**: molti pazienti, infatti, hanno prove allergometriche cutanee positive per molti allergeni e si pone dunque il problema di comprendere quali siano clinicamente rilevanti, ovvero correlati all’insorgenza dei sintomi, e quali invece siano trascurabili.

In casi dubbi o complicati, si procede quindi ai **test di secondo livello**: sono test effettuati sul siero del paziente, e che misurano i livelli ematici delle **IgE** (immunoglobuline E, la classe di anticorpi coinvolta nelle reazioni allergiche) verso un’intera **fonte allergica**, come per esempio i pollini o gli acari. Se però i risultati sono positivi, abbiamo sì individuato la fonte allergica, ma non la **proteina allergenica specifica implicata**. Alcune proteine (“allergeni”) sono infatti tipiche della fonte allergenica, mentre altre invece sono condivise con altre fonti. Questo pone la necessità di comprendere, ai fini della diagnosi, quali reattività siano genuine e quali invece siano ascrivibili al fenomeno della cosiddetta **cross-reattività**”.

Test per la diagnosi molecolare respiratoria e alimentare

Nuovo test per la diagnostica molecolare chiamato Allergy Explorer – ALEX, rappresenta un punto di svolta. Il test è infatti in grado di misurare 282 componenti contemporaneamente, di cui **125** sono singole **proteine**; con un **unico prelievo** di sangue quindi abbiamo ad oggi il più ampio risultato possibile in termini di componenti allergeniche valutate.

Nello studio pubblicato sul World Allergy Organization Journal è stato verificato che i risultati del test sono **ripetibili**, correlabili a quelli ottenuti con altri test sierici e dunque **attendibili**. In un’ottica di **medicina di precisione**, questo test è perfetto in ambito allergologico, perché è capace di individuare la vera causa della reazione allergica e scegliere la terapia più appropriata, per esempio in caso di immunoterapia.

Questo test si rivela prezioso anche per quanto riguarda il **rapporto tra allergia ai pollini e alimenti**, considerato che determinate proteine sono contenute in diverse fonti del mondo vegetale. Alcune proteine infatti – se ingerite – sono responsabili dello scatenarsi dei sintomi tipici delle allergie alimentari, mentre altre determinano la comparsa di sintomi lievi.

Anche in questo caso, sapere a quali proteine il paziente è allergico ci permette di stratificare il rischio della gravità dell’allergia e di fornire al paziente indicazioni più precise in termini di consumi alimentari, suggerendo quali alimenti può mangiare senza problemi e a quali invece

deve prestare attenzione perché la loro ingestione potrebbe determinare una reazione allergica e di che entità.

Allergy Explorer è il primo test allergologico in vitro multiplex che permette la misurazione simultanea delle IgEtotali (tIgE) e delle IgE specifiche (sIgE) per numerosi estratti allergenici e allergeni molecolari. Il protocollo di dosaggio ALEX integra un potente inibitore CCD (determinanti carboidratici cross-reattivi) durante l'incubazione del siero, rendendo così più chiari i risultati per le IgE specifiche. Ciò riduce l'onere dell'interpretazione per i medici dei pazienti positivi a i CCD e aumenta la specificità dei risultati del nostro test. La maggior parte dei preparati allergenici originati da piante o insetti contiene determinanti carboidratici cross-reattivi (CCD). Gli anticorpi IgE diretti verso i CCD mostrano una reattività crociata con tutte le proteine contenenti questi epitopi CCD. IgE anti-CCD sono presenti approssimativamente nel 25% dei pazienti, il che comporta un numero significativo di risultati di positività aspecifica sIgE, specialmente quando si testano gli estratti allergenici. Gli anticorpi anti-CCD sono stati ritenuti non significativi dal punto di vista clinico (Malanda in Hetal. 2007EurAnnAllergyClinImmunol).

Ottenere profili di sensibilizzazione esaustivi utilizzando i convenzionali sistemi di test singleplex (determinazione di un 1 solo analita per dosaggio ad es. un allergene) può risultare laborioso. Spesso sono richiesti diversi cicli di test per arrivare ad una diagnosi chiara e le IgE totali devono essere esaminate separatamente. ALEX offre invece un quadro pressoché completo della situazione del paziente, IgE totali comprese, rendendo più efficiente questo approccio, altrimenti frammentato. A disposizione ci sono oltre 150 estratti allergenici e oltre 100 allergeni molecolari. Allergeni molecolari disponibili in esclusiva includono marcatori di rischio della famiglia delle proteine di accumulo e altri nuovi marcatori (ad es. acari della polvere di casa, malassezia sympodialis).

Il test ALEX è destinato a supportare la diagnosi di malattie allergiche in associazione ad altri riscontri diagnostici quali anamnesi, storia clinica o esame obiettivo. Oltre ad identificare le fonti allergeniche sensibilizzanti, fornisce un profilo IgE molecolare pressoché completo ad alta risoluzione. In confronto alla sola diagnostica basata sugli estratti, questo sistema può aggiungere informazioni diagnostiche rilevanti:

- Indicazioni per immunoterapia specifica
- Valutazione di rischio per il vostro paziente per evitare severe reazioni allergiche alimentari
- Informazioni molecolari sulla reattività crociata

L'immunoterapia specifica è un trattamento causale per le malattie allergiche, specialmente nelle allergie inalatorie e al veleno. Con un approccio diagnostico che utilizza soltanto gli estratti,

spesso il risultato del test indica positività a fonti allergeniche multiple: questi risultati possono indicare sia una vera co-sensibilizzazione, sia una sensibilizzazione crociata. A risolvere la questione intervengono gli allergeni molecolari. I test allergologici basati suglie stratti si sono dimostrati utili per l'identificazione della fonte allergenica responsabile. Tuttavia, solo aggiungendo le risposte agli antigeni molecolari si può avere un quadro completo e prendere la decisione terapeutica ottimale.

Ad esempio, le proteine di accumulo quali Arah1,2,3 o 6 possono causare sintomi allergici che possono arrivare fino allo shock anafilattico. Arah8, la proteina PR10, normalmente non è invece in grado di causare gravi problemi, ma dà comunque un risultato di positività al test con gli estratti generando così una potenziale incertezza sia per il medico che per il paziente. Scenari similari esistono per diversi altri allergeni quali soia, noci e nocciole.

Come si effettua il test?

Il test viene effettuato su un semplice prelievo di sangue. Non è necessaria alcuna preparazione specifica nei giorni precedenti, bastano le 8-10 di diguno fino al prelievo (è possibile bere acqua). Il processo di analisi e refertazione viene gestito nel rispetto delle modalità di trattamento dei dati sensibili secondo le linee guida del garante della privacy (dlg 196/03).

Quanto costa il test e quali sono i tempi di attesa?

Per conoscere il prezzo del test e i tempi di refertazione è possibile contattare la nostra segreteria al numero di tel. 0815304881 negli orari di ufficio: tutte le mattine lun-ven 7.30-10.30 / lun-mer-ven anche pomeriggio 16.00-18.30

Si allegano alla presente:

1. Elenco allergeni suddivisi in Estratto e Molecolare;
2. Articoli di rilievo (bibliografia)

ALEX® Lista Allergeni					
Componente/Estratto	**Codice allergene**	**Nome Comune**	**Nome Scientifico**	**Componente**	**Funzione**
Polline					
Graminacee					
E	g17	Erba bahia	Paspalum notatum		
E	g2	Gramigna Rossa	Cynodon dactylon		
E	g7	Cannuccia di palude	Phragmites communis		
E	g202	Polline mais	Zea mays		
E	g10	Sorghetta	Sorghum halepense		
C	g100	Polline	Lolium perenne	nLol p 1	Beta-Expansina
E	g12	Segale polline	Secale cereale		
E	g6	Fleo	Phleum pratense		
C	g205	Fleo	Phleum pratense	rPhl p 1	Beta-Expansina
C	g206	Fleo	Phleum pratense	rPhl p 2	Expansina
C	g215	Fleo	Phleum pratense	rPhl p 5.0101	Erba Gruppo 5/6
C	g209	Fleo	Phleum pratense	rPhl p 6	Erba Gruppo 5/6
C	g210	Fleo	Phleum pratense	rPhl p 7	Polcalcina
C	g212	Fleo	Phleum pratense	rPhl p 12	Profilina
Polline Alberi					
E	t19	Acacia	Acacia spp.		
E	t2	Ontano	Alnus glutinosa		
C	t100	Ontano	Alnus glutinosa	rAln g 1	PR-10
C	t101	Ontano	Alnus glutinosa	rAln g 4	Polcalcina
C	t226	Cipresso	Cupressus arizonica	nCup a 1	Pectate Liasi
E	t25	Frassino	Fraxinus excelsior		
C	t103	Frassino	Fraxinus excelsior	rFra e 1	Ole e 1-Famiglia
E	t5	Faggio	Fagus sylvatica		

E	t14	Pioppo	Populus nigra		
E	t222	Cipresso	Cupressus sempervirens L.		
C	t105	Palma da datteri	Phoenix dactylifera	nPho d 2	Profilina
E	t8	Olmo	Ulmus campestris		
E	t4	Polline nocciolo	Corylus avellana		
C	t102	Polline nocciolo	Corylus avellana	rCor a 1.0103	PR-10
E	t106	Lilla	Syringa vulgaris		
E	t11	Platano	Platanus acerifolia		
C	t241	Platano	Platanus acerifolia	rPla a 1	Pianta Invertasi
E	t63	Cedro Montano	Juniperus ashei		
E	t71	Mora	Morus rubra		
E	t7	Quercia	Quercus robur		
E	t9	Oliva	Olea Europaea		
C	t224	Oliva	Olea Europaea	nOle e 1	Olive Comuni Gruppo 1
C	t104	Oliva	Olea Europaea	rOle e 2	Profilina
E	t210	Ligustro	Ligustrum vulgare		
E	t3	Betulla bianca	Betula verrucosa		
C	t215	Betulla bianca	Betula verrucosa	rBet v 1	PR-10
C	t216	Betulla bianca	Betula verrucosa	rBet v 2	Profilina
C	t225	Betulla bianca	Betula verrucosa	rBet v 6	Isoflavon Reductasi
E	t17	Sugi	Cryptomeria japonica		
E	t10	Polline noce	Juglans regia		
Polline Erbe					
E	w101	Mercurialis Annua	Mercurialis annua		
E	w10	Farinello	Chenopodium album		
C	w100	Farinello	Chenopodium album	rChe a 1	Ole e 1-Famiglia
E	w6	Artemisia	Artemisia vulgaris		
C	w231	Artemisia	Artemisia vulgaris	rArt v 1.0101	Pianta Defensina
C	w233	Artemisia	Artemisia vulgaris	rArt v 3.0201	nsLTP Typ 1
E	w20	Ortica	Urtica dioica		
E	w14	Chenopodio	Amaranthus retroflexus		

E	w1	Ambrosia	Ambrosia artemisiifolia/elatior		
C	w230	Ambrosia	Ambrosia artemisiifolia/elatior	rAmb a 1	Pectate Liasi
C	w300	Ambrosia	Ambrosia artemisiifolia/elatior	rAmb a 4	Pianta Defensina
E	w9	Piantaggine lanciuola	Plantago lanceolata		
C	w234	Piantaggine lanciuola	Plantago lanceolata	rPla l 1	Ole e 1-Famiglia
E	w11	Kali turgida	Salsola kali		
E	w18	Romice acetosella	Rumex acetocella		
E	w21	Erba vetriola	Parietaria judaica		
C	w211	Erba vetriola	Parietaria judaica	rPar j 2	nsLTP Typ 1
			Muffe & Lieviti		
E	m6	Fungo	Alternaria alternata		
C	m229	Fungo	Alternaria alternata	rAlt a 1	Alt a 1-Famiglia
E	m3	Aspergillo	Aspergillus fumigatus		
C	m220	Aspergillo	Aspergillus fumigatus	rAsp f 3	Proteina Peroxisomale
C	m221	Aspergillo	Aspergillus fumigatus	rAsp f 4	Sconosciuto
C	m222	Aspergillo	Aspergillus fumigatus	rAsp f 6	Mn Superossido-Dismutasi
E	m5	Candida	Candida albicans		
E	m2	Fungo	Cladosporium herbarum		
C	m100	Fungo	Cladosporium herbarum	rCla h 8	Deidrogenasi Catena Corta
C	y1	Fungo	Malassezia sympodialis	rMala s 1	Sconosciuto
C	y2	Fungo	Malassezia sympodialis	rMala s 5	Cyclofilina
C	y3	Fungo	Malassezia sympodialis	rMala s 6	Sconosciuto
C	y4	Fungo	Malassezia sympodialis	rMala s 9	Mn Superossido-Dismutasi
C	y5	Fungo	Malassezia sympodialis	rMala s 11	Sconosciuto
E	m1	Fungo	Penicilium chrysogenum		
			Acari e Scarafaggi		
E	d70	Acaro	Acarus siro		
E	i206	Scarafaggio	Periplaneta americana		
C	i300	Scarafaggio	Periplaneta americana	rPer a 7	Tropomiosina

E	d2	Acaro	Dermatophagoides farinae		
C	d100	Acaro	Dermatophagoides farinae	rDer f 1	Cisteina Proteasi
C	d101	Acaro	Dermatophagoides farinae	rDer f 2	Famiglia NPC2
E	d201	Acaro	Blomia tropicalis		
E	d1	Acaro	Dermatophagoides pteronyssinus		
C	d202	Acaro	Dermatophagoides pteronyssinus	rDer p 1	Cisteina Proteasi
C	d203	Acaro	Dermatophagoides pteronyssinus	rDer p 2	Famiglia NPC2
C	d103	Acaro	Dermatophagoides pteronyssinus	rDer p 5	Sconosciuto
C	d104	Acaro	Dermatophagoides pteronyssinus	rDer p 7	Polvere Gruppo 7
C	d205	Acaro	Dermatophagoides pteronyssinus	rDer p 10	Tropomiosina
C	d102	Acaro	Dermatophagoides pteronyssinus	rDer p 11	Miosina, catena pesante
K	d209	Acaro	Dermatophagoides pteronyssinus	rDer p 23	Chitiniasi classe III, Peritrofin-tipo Proteina Dominio
E	i6	Scarafaggio	Blatella germanica		
C	i100	Scarafaggio	Blatella germanica	rBla g 1	Scarafaggio Gruppo 1
C	i101	Scarafaggio	Blatella germanica	rBla g 2	Aspartyl proteasi
C	i102	Scarafaggio	Blatella germanica	rBla g 4	Lipocalina
C	i103	Scarafaggio	Blatella germanica	rBla g 5	Glutathione S-transferasi
E	d73	Acaro	Glycyphagus domesticus		
C	d105	Acaro	Glycyphagus domesticus	rGly d 2	Famiglia NPC2
E	d71	Acaro	Lepidoglyphus destructor		
E	d72	Acaro	Tyrophagus putrescentiae		

Pelle Animale					
E	e1	Gatto	Felis domesticus		
C	e94	Gatto	Felis domesticus	rFel d 1	Uteroglobina
C	e220	Gatto	Felis domesticus	nFel d 2	Serum Albumina
C	e228	Gatto	Felis domesticus	rFel d 4	Lipocalina
E	e4	Pelle Mucca	Bos domesticus		
C	e100	Carne Mucca	Bos domesticus	rBos d 2	Lipocalina
E	e5	Cane	Canis familiaris		
C	e101	Cane	Canis familiaris	rCan f 1	Lipocalina
C	e102	Cane	Canis familiaris	rCan f 2	Lipocalina
C	e221	Cane	Canis familiaris	nCan f 3	Serum Albumina
E	e80	Pelle Capra	Capra hircus		
E	e6	Porcellino d'India	Cavia porcellus		
E	e84	Criceto	Cricetus cricetus		
E	e3	Pelle Cavallo	Equus caballus		
C	e227	Pelle Cavallo	Equus caballus	rEqu c 1	Lipocalina
C	e103	Topo Casalingo	Mus musculus	nMus m 1	Lipocalina
E	e83	Maiale	Sus domesticus		
E	e82	Pelle Coniglio	Oryctolagus spp.		
E	e73	Topo	Rattus norvegicus		
E	e81	Pelle Pecora	Ovis aries		
Hymenoptera Veleno					
E	i3	Veleno Vespa	Vespula vulgaris		
C	i209	Veleno Vespa	Vespula vulgaris	rVes v 5	Antigene 5
E	i1	Veleno Ape	Apis mellifera		
C	i208	Veleno Ape	Apis mellifera	r/nApi m 1	Fosfolipasi A2
C	i214	Veleno Ape	Apis mellifera	rApi m 2	Ialuronidasi
C	i217	Veleno Ape	Apis mellifera	rApi m 10	Icarapina Variante 2
E	i25	Calabrone	Dolichovespula spp.		
E	i4	Veleno Calabrone	Polistes dominulus		
C	i210	Veleno Calabrone	Polistes dominulus	rPol d 5	Antigene 5

Cibo					
Cereali & Semi					
E	f6	Orzo	Hordeum vulgare		
E	f11	Grano Saraceno	Fagopyrum esculentum		
C	f508	Grano Saraceno	Fagopyrum esculentum	nFag e 2	2S Albumina
E	f8	Mais	Zea mays		
E	f5	Segale coltivata	Secale cereale		
E	f335	Semi Lupino	Lupinus albus		
E	f55	Miglio	Panicum miliaceum		
E	f7	Avena	Avena sativa		
E	f224	Semi Papavero	Papaver somniferum		
C	f516	Semi Papavero	Papaver somniferum	nPap s 2S Albumin	2S Albumina
E	f226	Semi Zucca	Cucurbita pepo		
E	f347	Quinoa	Chenopodium quinoa		
E	f9	Riso	Oryza sativa		
E	f10	Sesamo	Sesamum indicum		
C	f518	Sesamo	Sesamum indicum	nSes i 1	2S Albumina
E	k84	Semi girasole	Helianthus annuus		
E	f124	Farro	Triticum spelta		
E	f4	Grano	Triticum aestivum		
C	f98	Grano	Triticum aestivum	nTri a Gliadin	Gliadina
Uova e Latte					
E	f1	Albume	Gallus domesticus		
C	f233	Albume	Gallus domesticus	nGal d 1	Ovomucoide
C	f232	Albume	Gallus domesticus	nGal d 2	Ovalbumina
C	f323	Albume	Gallus domesticus	nGal d 3	Ovotransferrina
C	k208	Albume	Gallus domesticus	nGal d 4	Lysozyma C
C	f510	Tuorlo	Gallus domesticus	nGal d 5	Serum Albumina
E	f75	Tuorlo	Gallus domesticus		
cam	f506	Cammello	Camelus dromedarius		

E	f2	Latte Mucca	Bos domesticus		
C	f76	Latte Mucca	Bos domesticus	nBos d 4	α-Lactoalbumina
C	f77	Latte Mucca	Bos domesticus	nBos d 5	β-Lactoglobulina
C	f78	Latte Mucca	Bos domesticus	nBos d 8	Caseina
E	f300	Latte Capra	Capra hircus		
E	f286	Latte Cavallo	Equus caballus		
E	f325	Latte Pecora	Ovis aries		
Frutta					
E	f49	Mela	Malus domestica		
C	f434	Mela	Malus domestica	rMal d 1	PR-10
C	f514	Mela	Malus domestica	nMal d 2	TLP
C	f435	Mela	Malus domestica	rMal d 3	nsLTP Typ 1
E	f92	Banana	Musa acuminata		
E	f288	Mirtillo	Vaccinium myrtillus		
E	f242	Ciliegia	Prunus spp.		
E	f328	Fico	Ficus carica		
C	f521	Uva	Vitis vinifera	nVit v 1	nsLTP Typ 1
E	f84	Kiwi	Actinidia deliciosa		
C	f500	Kiwi	Actinidia deliciosa	nAct d 1	Cisteina Proteasi
C	f503	Kiwi	Actinidia deliciosa	nAct d 2	TLP
C	f501	Kiwi	Actinidia deliciosa	nAct d 5	Kiwellina
C	f502	Kiwi	Actinidia deliciosa	nAct d 10	nsLTP Typ 1
E	f348	Litchi	Litchi chinesis		
E	f91	Mango	Mangifera indica		
E	f87	Melone	Cucumis melo		
E	f33	Arancia	Citrus sinensis		
E	f293	Papaya	Carica papaya		
E	f95	Pesca	Prunus persica		
C	f420	Pesca	Prunus persica	n/r Pru p 3	nsLTP Typ 1
E	f94	Pera	Pyrus communis		
E	f255	Prugna	Prunus domestica		

E	f343	Lampone	Rubus idaeus		
E	f44	Fragola	Fragaria ananassa		
Legumi e Frutta Secca					
E	f20	Mandorla	Prunus dulcis		
E	f18	Noce brasiliana	Bertholletia excelsa		
C	f354	Noce brasiliana	Bertholletia excelsa	nBer e 1	2S Albumina
E	f202	Anacardo	Anacardium occidentale		
C	f443	Anacardo	Anacardium occidentale	rAna o 3	2S Albumina
E	f309	Ceci	Cicer arietinus		
E	f17	Nocciola	Corylus avellana		
C	f428	Nocciola	Corylus avellana	rCor a 1.0401	PR-10
C	f425	Nocciola	Corylus avellana	rCor a 8	nsLTP Typ 1
C	f440	Nocciola	Corylus avellana	nCor a 9	11S Globulina
C	f522	Nocciola	Corylus avellana	nCor a 11	7/8S Globulina
C	f439	Nocciola	Corylus avellana	nCor a 14	2S Albumina
E	f235	Lenticchie	Lens culinaris		
E	f345	Noce Macadamia	Macadamia integrifolia		
C	f513	Noce Macadamia	Macadamia integrifolia	nMac i 2S Albumin	2S Albumina
E	f12	Piselli	Pisum sativum		
E	f13	Arachide	Arachis hypogea		
C	f422	Arachide	Arachis hypogea	nAra h 1	7/8S Globulina
C	f423	Arachide	Arachis hypogea	rAra h 2	2S Albumina
C	f424	Arachide	Arachis hypogea	nAra h 3	11S Globulina
C	f447	Arachide	Arachis hypogea	nAra h 6	2S Albumina
C	f352	Arachide	Arachis hypogea	rAra h 8	PR-10
C	f427	Arachide	Arachis hypogea	rAra h 9	nsLTP Typ 1
E	f201	Noce Pecan	Carya illinoensis		
E	f203	Pistacchio	Pistacia vera		
E	f14	Soia	Glycine max		
C	f353	Soia	Glycine max	rGly m 4	PR-10

C	f431	Soia	Glycine max	rGly m 5	7/8S Globulina
C	f432	Soia	Glycine max	nGly m 6	11S Globulina
C	f511	Soia	Glycine max	nGly m 8	2S Albumina
E	f256	Noce	Juglans regia		
C	f441	Noce	Juglans regia	nJug r 1	2S Albumina
C	f512	Noce	Juglans regia	nJug r 2	7/8S Globulina
E	f315	Fagiolo bianco	Phaseolus vulgaris		
Pesce & Frutti di mare					
C	p10	Verme aringa	Anisakis simplex	rAni s 1	Kunitz Serin Proteasi Inibitore
C	p11	Verme aringa	Anisakis simplex	rAni s 3	Tropomiosina
E	f3	Merluzzo	Gadus morhua		
C	f509	Merluzzo	Gadus morhua	nGad m 1	β-Parvalbumina
C	f355	Carpa	Cyprinus carpio	rCyp c 1	β-Parvalbumina
E	f37	Cozza	Mytilus edulis		
E	f23	Granchio	Chionoecetes spp.		
E	f258	Calamaro	Loligo spp.		
E	f80	Aragosta	Homarus gammarus		
E	f290	Ostrica	Ostrea edulis		
E	f41	Salmone	Salmo salar		
E	f338	Capasanta	Pecten spp.		
E	f515	Gamberetto	Pandalus borealis		
E	f24	Gamberetto	Litopaenaeus setiferus, Farfantepenaeus aztecus, Farfantepenaeus dourarum		
C	f517	Gamberetto	Penaeus monodon	nPen m 1	Tropomiosina
E	f40	Tonno	Thunnus albacares		
E	f207	Mollusco	Ruditapes spp.		
Spezie					
E	f271	Anice	Pimpinella anisum		
E	f265	Cumino	Carum carvi		
E	f89	Mostarda	Brassica / Sinapis spp.		

C	f519	Mostarda	Brassica / Sinapis spp.	nSin a 1	2S Albumina
E	f283	Origano	Origanum vulgare		
E	f218	Paprika	Capsicum annuum		
E	f86	Prezzemolo	Petroselinum crispum		
Carne					
E	f27	Carne Mucca	Bos domesticus		
C	e204	Carne Mucca	Bos domesticus	nBos d 6	Serum Albumina
E	f83	Carne Pollo	Gallus domesticus		
E	f321	Carne Cavallo	Equus caballus		
E	f88	Carne Pecora	Ovis aries		
E	f26	Porco	Sus domesticus		
E	f213	Carne Coniglio	Oryctolagus spp.		
E	f284	Tacchino	Meleagris gallopavo		
Vegetali e Funghi					
E	f96	Avocado	Persea americana		
E	f216	Cavolo	Brassica oleracea var. Capitata		
E	f31	Carota	Daucus carota		
C	f507	Carota	Daucus carota	rDau c 1	PR-10
E	f85	Sedano	Apium graveolens		
C	f417	Sedano	Apium graveolens	rApi g 1	PR-10
C	f504	Sedano	Apium graveolens	rApi g 2	nsLTP Typ 1
C	f505	Sedano	Apium graveolens	rApi g 6	nsLTP Typ 2
E	f47	Aglio	Allium sativum		
E	f215	Lattuga	Lactuca sativa		
E	f342	Olive	Olea europaea		
E	f48	Cipolla	Allium cepa		
E	f35	Patata	Solanum tuberosum		
E	f25	Pomodoro	Solanum lycopersicum		
C	f520	Pomodoro	Solanum lycopersicum	nSola l 6	nsLTP Typ 2
E	f212	Fungo Bianco	Agaricus hortensis/bisporus		
E	f324	Luppolo	Humulus lupulus		

E	f45	Lievito	Saccharomyces cerevisiae		
Altri					
E	k82	Lattice	Hevea brasiliensis		
C	k215	Lattice	Hevea brasiliensis	rHev b 1	Fattore di allungamento della Gomma
C	k217	Lattice	Hevea brasiliensis	rHev b 3	Particella proteica di piccola Gomma
C	k218	Lattice	Hevea brasiliensis	rHev b 5	Sconosciuto
C	k220	Lattice	Hevea brasiliensis	rHev b 6.02	Pro-Hevein
C	k221	Lattice	Hevea brasiliensis	nHev b 8	Profilina
C	k224	Lattice	Hevea brasiliensis	rHev b 11	Chitinasi Classe 1
E	k81	Fico Beniamino	Ficus benjamina		
C	k202	Ananas	Ananas comosus	nAna c 2	CCD
C	o214	Hom s Lattoferrina		rHom s LF	CCD

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Heffler et al. World Allergy Organization Journal (2018) 11:7
https://doi.org/10.1186/s40413-018-0186-3

World Allergy Organization Journal

ORIGINAL RESEARCH Open Access

Extended IgE profile based on an allergen macroarray: a novel tool for precision medicine in allergy diagnosis

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Abstract

Background: Precision medicine (PM) is changing the scope of allergy diagnosis and treatment. An in vitro IgE assay, a prototype PM method, was developed in the sixties and has garnered increasing interest because of the introduction of recombinant components in the test. More recently, microarrays of allergen components have significantly improved the ability to describe the IgE profile. Aim of this study was to evaluate the characteristics of the newly developed Allergy Explorer (ALEX), a macroarray containing both extracted "whole" allergens and molecular components. This method allows the acquisition of an IgE profile comprising 282 reagents (157 allergen extracts and 125 components), resulting in the widest screening of potential allergens available.

Methods: Sera from 43 patients with allergies were assayed with ALEX and then with ImmunoCAP ISAC. The results of the two tests were compared, and the consistency of the molecular results with the presence of IgE in the relevant extract was also evaluated.

Results: A good correlation between ISAC and ALEX was observed. The ALEX results for second-level tests (i.e., specific IgE to complete extracted allergens) were consistent with the results obtained for the relevant components.

Discussion: Despite differences in the methodology, the IgE profiles detected for molecular allergens by ALEX and ISAC were very similar. The differences were mainly related to the lower dynamic range of ALEX and to the use of a CCD inhibitor in the first incubation phase, which reduced the binding of IgE to CCD, as represented in the extracted allergens and components.

Conclusion: Based on our findings, ALEX is a novel tool for describing the IgE profile in a PM setting, where the IgE assay must be performed on many allergens and components. In particular, polysensitized patients and patients with pollen-food syndrome will have a real advantage due the combination of the second and third levels of allergy diagnostics in the same chip.

Keywords: Allergen extract, Allergen component, ISAC, ALEX, IgE assay, Laboratory methods

Background

Precision medicine (PM) has a relevant impact on many human sciences and a special impact on the diagnosis and treatment of allergic diseases [1]. Indeed, since its origin, in vivo and in vitro diagnostics have facilitated the accurate and personal treatment of the patient, resulting in a sort of progenitor of PM [2, 3]. Specific IgE analysis was developed in the sixties [4], but in the early nineties, a number of molecular allergens, cloned or obtained by biochemical purification [5], have significantly improved the quality of allergy diagnostics. Indeed, genuine sensitization is identified by the detection of IgE specific for components restricted to a given allergen. A cross-reaction is detected by the presence of an immune response to cross-reacting components, such as profilins and PR-10 [6]. In addition, molecular allergy

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diagnosis (MAD) allows the detection of IgE specific for "potentially dangerous" components (such as lipid transfer proteins) or apparently safe (or minimally dangerous) components, such as profilins and polcalcins [7]. International guidelines [8] still indicate that clinical history, physical examination and the Skin Prick Test (SPT) are the starting procedures (first level) of every allergy diagnosis (a top-down approach). Specific IgE assay performed on extracted (whole) allergens is considered a second-level diagnostic measure, and MAD is considered a third-level diagnostic [7, 9]. However, other authors suggested that a bottom-up diagnostic approach may also have advantages [10]. In this context, wide IgE profiling based on an allergen microarray (AMA) could be extremely useful. AMA was developed in early 2000 [11], and currently, ImmunoCAP ISAC (Thermo Fisher), based on 112 different molecular components (both extracted and recombinant), is the most studied and most frequently used molecular diagnostic tool based on a microarray [12]. A chip combining second- and third-level diagnostics has recently been developed by MacroArrayDX (Wien, Austria). This chip contains 157 allergen extracts and 125 molecular components and seems to be the widest allergen array currently available. In addition, basic IgE analysis on the allergen extracts is combined in the same test with the evaluation of IgE directed to relevant specific and cross-reactive components. Finally, the inhibition of CCD reactivity further improves the specificity of the IgE assay [13]. In the present study, we describe how this extended IgE profile can be considered a promising tool to support strategies of diagnosis and the treatment of modern PM in in allergic patients.

Methods

ALEX was developed by MacroArrayDX (Wien, Austria). This array contains 282 reagents (157 extractive allergens and 125 molecular components). The large majority of inhalant, food, latex and Hymenoptera allergen families are represented (Table 1). The test is commercially available, having attained CE certification, which, based on the Council Directive 93/42/EEC concerning medical devices [14], assures that the quality of the assay (i.e., limit of detection, precision and repeatability, absence of possible interferences caused by hemolysis and high levels of triglycerides, absence of an effect of high levels of total IgE, specificity and linearity) is in line with in vitro diagnostic (IVD) features. The different allergens and components are spotted onto a nitrocellulose membrane in a cartridge chip, which is then incubated with 0.5 mL of a 1:5 dilution of serum under agitation. Notably, the serum diluent contains a CCD inhibitor. After incubation for two hours, the chips are extensively washed, and a pretitered dilution of anti-human IgE labeled with alkaline phosphatase is added and incubated for 30 min. Following another cycle of extensive washing, the enzyme substrate is added, and after a few minutes, the reaction is complete. The membranes are dried, and the intensity of the color reaction for each allergen spot is measured by a CCD camera. The dedicated software digitalizes the images and prepares a report that lists the allergens and components and their score in kU_A/mL. Total IgE is also measured. Finally, an arbitrary calibration curve is obtained by reacting four spots with decreasing concentrations of specific IgE corresponding to $< 0.3\ kU_A/L$, $0.3 - 1\ kU_A/L$, $1 - 5\ kU_A/L$, $5 - 15\ kU_A/L$ and $> 15\ kU_A/mL$.

For the evaluation of the IgE profile in sera from patients with allergies, forty-three serum samples were analyzed by the novel assay. The sample size was calculated considering that, in preliminary assays, 12% of the allergens tested (including low score results) were different when assayed with ALEX and when assayed with other methods (such as specific IgE for extracts or components). Starting from this prestudy evidence, with a confidence level of 95% and a standard error of 0.05, the calculated sample size resulted in 43 different sera. Due to the large

Table 1 Composition of the allergens available on ALEX

	Total number	Number of extracts	Number of molecular components		Total number	Number of extracts	Number of molecular components
Animals	6	5	2	Fishes	5	3	2
CCD	1	1	2	Foods	23	17	6
Grasses	26	13	13	Fruits	28	21	7
Mites	24	9	15	Legumes	4	4	0
Molds	11	6	10	Meats	0	0	0
Pets	10	3	6	Milks	11	6	5
Trees	25	14	10	Seeds	27	10	17
Weeds	22	15	7	Shellfishes	10	9	1
Eggs	7	2	5	Latex	7	1	6
Extras	21	14	6	Venoms	9	4	5

amount of allergen families (perennial or seasonal inhalants, food, etc.) and the virtual impossibility of studying all the possible families in a single work, sera with certain characteristics were selected. Therefore, samples from patients with a known reactivity to grasses (where the largest number of molecular components was available) and cross-reacting components, particularly PR-10, profilins and LTPs, were used [15]. In this context, it was considered that the added value of molecular diagnostics could be extensively described. All sera were previously tested with ImmunoCAP ISAC. Since ALEX is a commercially available method, patients (from the private medical practice of one of the authors) were warned that their serum would be tested, without cost, with another method that could define their IgE profile in an exhaustive way. All patients accepted the proposal verbally. The following parameters were evaluated: a) correlation between the results of extracts and the results of relevant components in ALEX; b) correlation between the results of ALEX and the results of ImmunoCAP ISAC; c) correlation between the sum of the scores of ISAC and the sum of the scores of ALEX. The second and the third parameters were assayed for only the components represented in both reagents.

Statistical analysis was performed by using the statistical routines of Microsoft Excel and PAST v3.16, a free software for scientific analysis.

Results

a) Analysis of the consistency of the ALEX results. This analysis was performed using patient sera to identify situations in which the extracts were positive but the component result was negative. A clear consistency was detected for kiwi, alder, ragweed, celery, peanut, mugwort, *Aspergillus fumigatus*, birch, dog, hazel, *Dermatophagoides pteronyssinus, D. farinae*, cat, codfish, hen egg, apple, wall pellitory, timothy grass, peach, and ash (Fig. 1). Poor consistency was observed for Hevea b., where extracts were negative but Hev b 8 (a profilin) was positive in some patients.

b) Comparison with the results of ImmunoCAP ISAC. For this aim, two comparisons were made: first, a comparison of the components present in both assays (ISAC and ALEX) and represented in a suitable number in the cohort of patients evaluated, and second, a comparison of the capacity of identifying the same component families. The results of the single-component comparisons are shown (Fig. 2). It is evident that the coefficients of correlation were highly significant for every comparison. Indeed, for this number of comparisons, a value $R > 0.39$ corresponds to a probability of 0.01% for "absence of correlation", and the lowest value observed was 0.51 for Jug r 2, where the use of the CCD inhibitor in the sample diluent modified the reactivity to a well-known highly glycosylated component [16]. A similar result was achieved by comparing the results by ROC curves (not shown). It is evident that the results closer to the upper left corners indicated that the prediction of both methods was highly comparable.

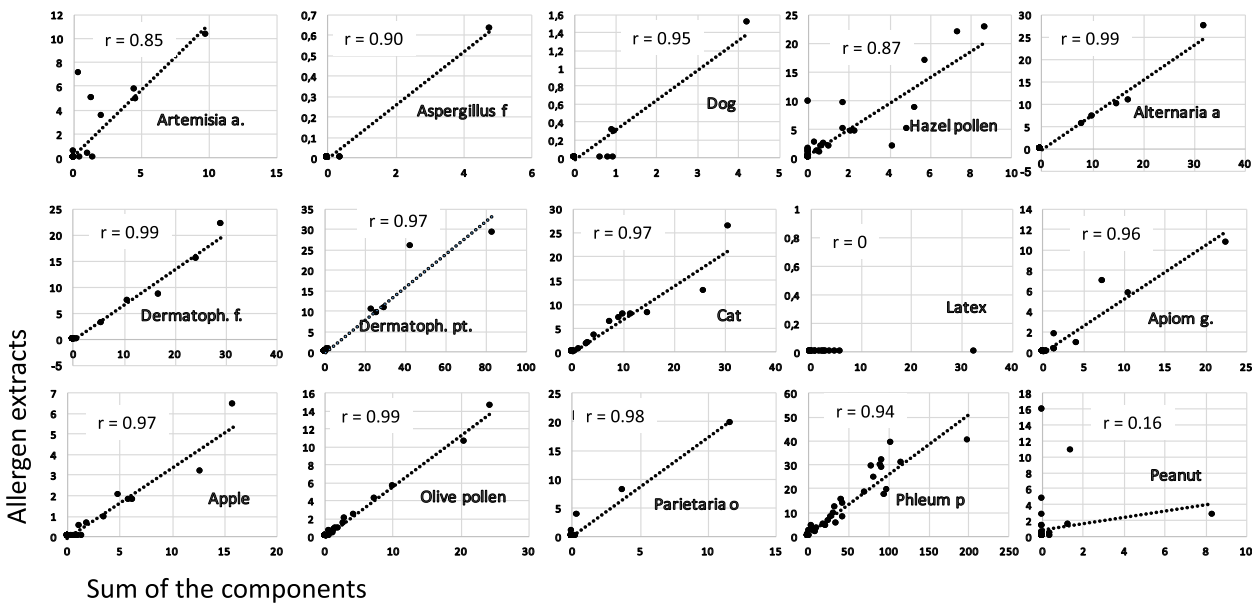

Fig. 1 Correlation between the sum of the components (horizontal axis) and the results of the relevant allergen extracts (vertical axis) obtained by ALEX

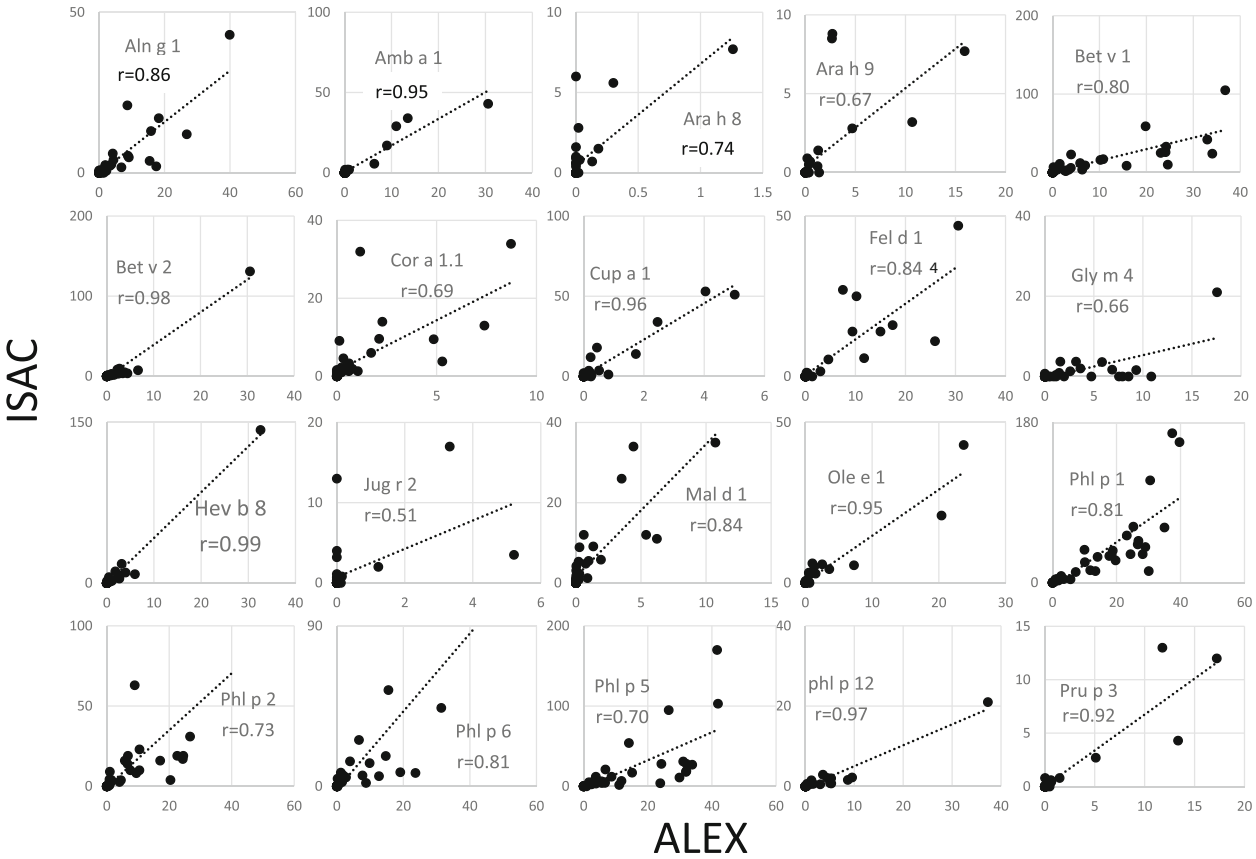

Fig. 2 Correlation between the results obtained by ALEX (horizontal axis) and the results obtained by ISAC (vertical axis)

c) Regarding the capacity of ALEX to identify component families, compared to the capacity of ISAC, a statistical analysis was performed, and the results are shown in Table 2. It is evident that certain heterogeneity can be observed, particularly in the frequency of positive results within the analyzed population. For example, the frequency of positive results is higher using ISAC for LTPs, PR-10, profilins and 2S albumins, while ALEX is more frequently positive for tropomyosins, 11S and 7S globulins. Consistently, the dynamic range of ISAC appears, to some extent, to be higher than that of ALEX, at least for certain component families, such as LTPs.

d) Another comparison was made by plotting the results of components present in both ALEX and ISAC in the same patient. Figure 3 shows the evaluation of 12 patients representative of the patient cohort. A significant correlation ($r > 0.39$, $p > 0.01$) was observed in 10 out of 12 patients. In a single patient (identified by K), the correlation coefficient R was 0.38 ($p < 0.02$), and in only a single patient (L) could any correlation be observed. However, in these patients, the scores were extremely low and below any clinical or laboratory significance.

e) Finally, the effect of the CCD inhibitor was evaluated in some representative samples. Figure 4 shows the ALEX raw data on the macroarray. It is evident that the treatment of sera by the CCD inhibitor results in a sharp decrease of the reactivity to the allergen extracts whose mixture of allergens is characterized by a high concentration of carbohydrate chains in the protein structure. This finding is also true for certain non-recombinant components [13, 17].

Discussion

In the absence of a gold standard for the evaluation of the performance of an assay of specific IgE, any correlation between different methods should be carefully evaluated. Indeed, the SPT cannot be used to evaluate the results of any serological assay, as it may be positive even in the absence of specific IgE. Specific IgE (assayed on the whole extract) cannot be directly compared with specific IgE measured from the molecular components: indeed, the positivity to the whole extract only rarely corresponds to the positivity of all the relevant

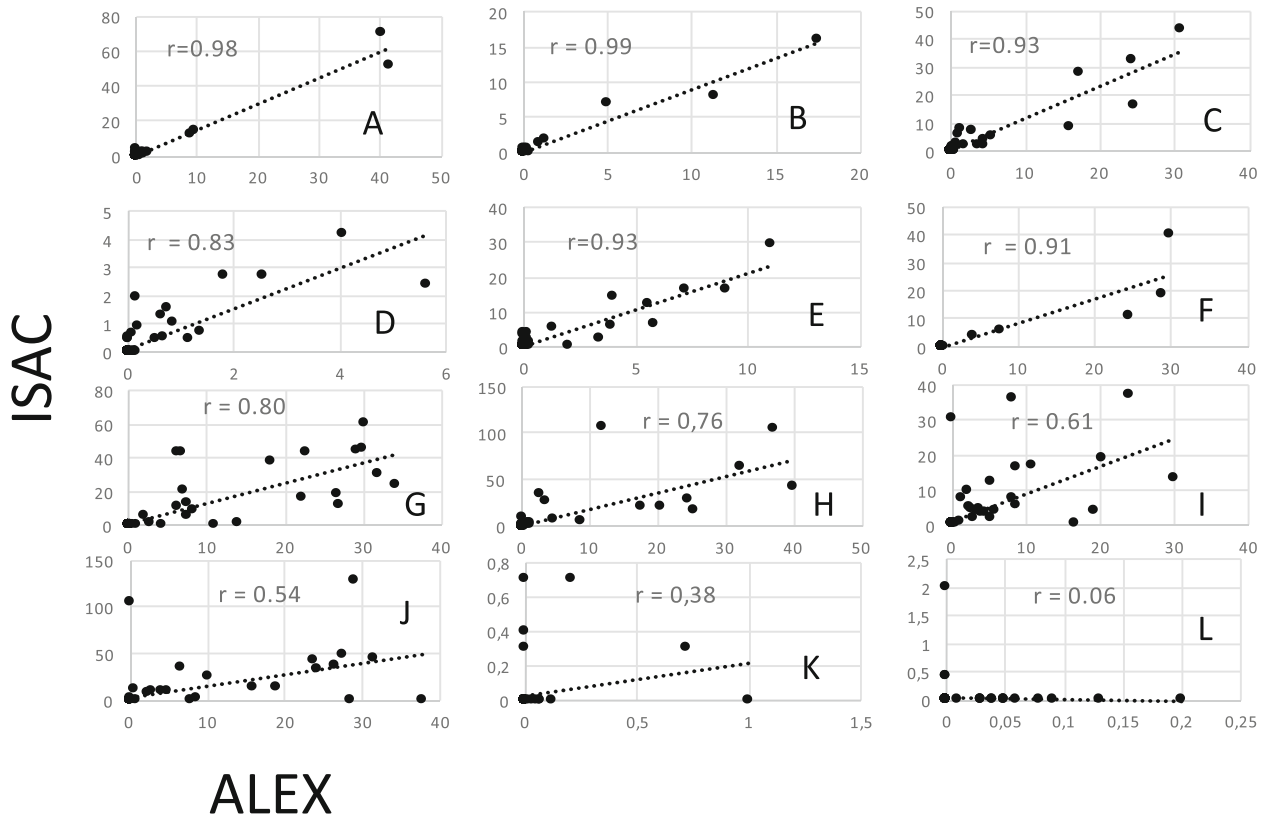

Fig. 3 Correlation between the results obtained by ALEX and ISAC at the patient level

components available [12]. Finally, the comparison of assays where recombinant molecules are used may have some pitfalls. Indeed, the clones used to produce the reagents are sometimes different, the folding of these molecules could be different, the immunosorbent used to adhere the component to the solid phase could interfere with the availability of certain epitopes, and finally, specific IgE molecules from different patients show different binding capacities to different epitopes [17]. Moreover, every immunoassay is based on specific concentrations of antigens, test sera, enzyme-labeled antisera and enzyme substrates suitable to offer the best dynamic range under the analytical conditions used. In allergy diagnostics, different platforms and substrates are currently used, and it is normal in laboratory medicine to observe that different serological assays generate different results, even if a correlation is frequently observed under certain operative conditions. In addition, the more sophisticated the assay (or the more complex the antigen or mixture of antigens) is, the greater the heterogeneity of the results.

Table 2 Comparison of percent of positive and mean value in kUA/L for a panel of relevant cross-reacting components assayed by ALEX and by ISAC

	ALEX		ISAC	
Component family	% of positive	Mean value	% of positive	Mean value
LTPs	8.4%	0.43 kUA/L	11.9%	0.71 ISU
PR-10	28.9%	2.06 kUA/L	41.5%	2.84 ISU
Profilins	28.3%	1.46 kUA/L	34.8%	1.43 ISU
Tropomyosins	6.1%	1.44 kUA/L	3.3%	1.11 ISU
11S globulins	2.6%	0.038 kUA/L	0.8%	0.014 ISU
2S albumins	1.0%	0.012 kUA/L	1.5%	1.2 ISU
7S globulins	6.5%	0.16 kUA/L	2.6%	0.30 ISU

Having in mind these concepts, in the present study, we analyzed the capacity of ALEX, a novel tool that could be properly used in the bottom-up strategy of allergy diagnostics, to detect sensitization to allergens and components.

To validate ALEX performances, it was considered that this assay was developed on nitrocellulose as an immunosorbent, and the ligation of the allergen to the solid phase was performed by a nanoparticle. Therefore, within the same assay, a comparison of results from the whole extract and the results from relevant allergen-specific components could be accurately performed. A significant correlation between the results of whole extracts with those of the relevant components was

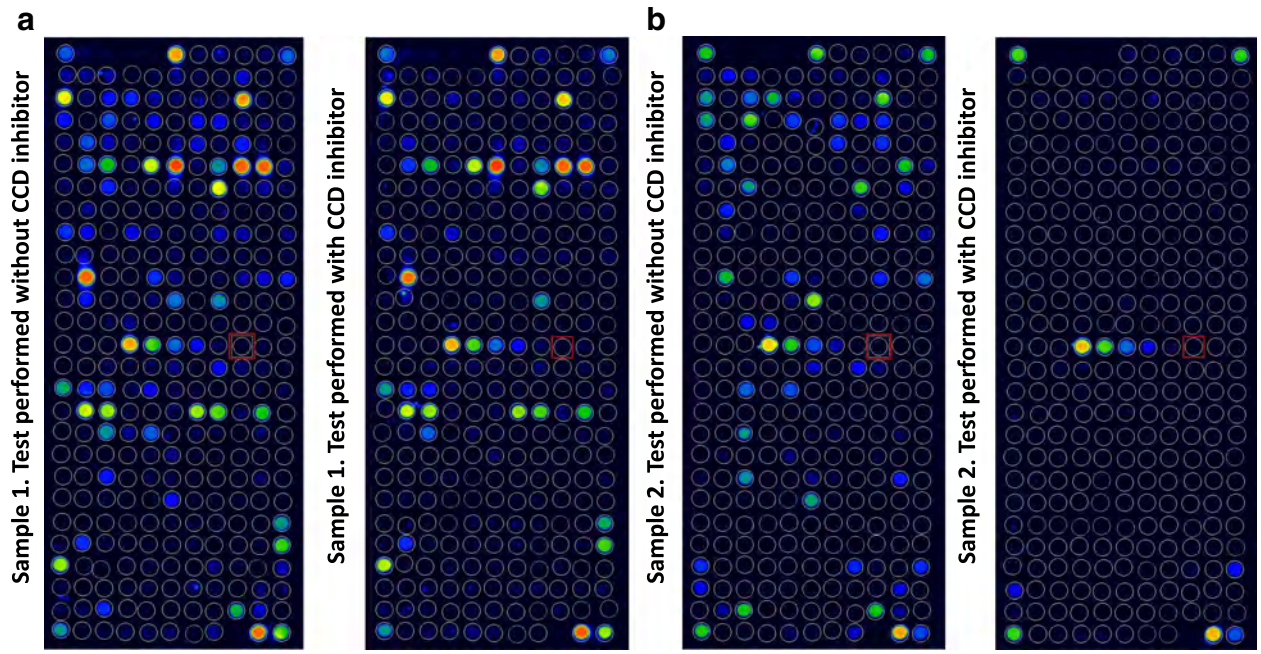

Fig. 4 Effects of CCD inhibition on two serum samples assayed by ALEX with or without CCD inhibition. It is evident that after the inhibition of CCD reactivity, the number and the intensity of spots on the chip was strongly reduced in panel **B** (only two spots excluding the calibration curve remained positive). In panel **A**, a less extreme situation was present, where the number and the intensity of spots was reduced but a large number were positive

observed. This finding is particularly interesting because, in the past, this strict correlation was not completely observed [12]. Some potential explanations for this result include a) the use of the same immunosorbent; b) the choice of representative components; and c) the use of a CCD inhibitor that reduces the non-specific recognition of IgE. The fact that, in a single assay, the allergist can detect positivity to a single extract and obtain information on the relevant components is a real added value.

When molecular components on ALEX were compared with the same components on ISAC, it was considered that the solid phases were different, the serum dilutions were different, the second antibody was probably different, and the enzyme substrate was also different. Additionally, ALEX uses a CCD inhibitor while ISAC does not. Nevertheless, laboratory methods are "artificial" procedures that attempt to mimic in vitro what is suspected to occur in vivo and, more importantly, the results from in vitro tests are used to support the allergist's diagnosis and therapy. However, despite technical differences, a significant correlation between methods should be achieved. At the component level, the correlation between the results of ALEX and those of ISAC was more than positive, at least for the IgE profiles used in the present preliminary study. Indeed, we focused on samples characterized by a strong IgE reaction against pollens and related cross-reacting allergens because this is an area in which molecular diagnostics seems to offer the most useful results [15]. All the correlations were significant, even if ISAC showed a wider dynamic range. The differences in the dynamic range should be discussed. Indeed, despite decades of using specific IgE in the clinic, the direct correlation between the specific IgE level and the severity of the disease has been observed for certain food allergens in single-plex assays [18]. However, for multi-plexed assays, this correlation has never been described as a rule for all allergens and does not seem to have a proven value in the clinic. On the basis of the observed results, it cannot be concluded that differences in the dynamic range have a significant effect on the performances of the assays.

The capacity to detect sensitization to component families was characterized by a certain heterogeneity. Possible explanations are that at the component level, different molecules were used in the two methods, resulting in a different capacity of sera to recognize different epitopes. In addition, the use of a CCD-inhibitor in ALEX may generate further differences. Finally, the strict correlation between the results of molecular components at a single patient level is the final evidence that ALEX performs similarly to ISAC.

The role of the CCD inhibitor is interesting [13]. Allergists are arguing the role of CCD in human pathology. From an analytical point of view, cross-reactions to CCD are frequent and could impact the decision to start a specific AIT [9, 19, 20]. Thus, the presence of a CCD inhibitor

allows a positive result only when the recognition of the allergen (or the component) is specific for the protein itself.

One of the principal added values of ALEX is its capacity to provide results on whole extracts and relevant components within the same assay. The combination of second- and third-level assays in the same test allows us to define, in a single hit, the presence of IgE sensitization and whether the reaction is genuine or cross-reactive. Considering the overall social and personal costs, the availability of all the results in a single analytical session has unequivocal advantages. This seems particularly interesting considering that the raw cost of a single allergen or component on the ALEX chip is approximately 0.30 €. Despite the fact that this may be disturbing for some allergists [21], the advantage of having a wide array of allergens and components also allows them to manage the patient using a bottom-up strategy: in this context, 282 allergens in a single chip facilitated an assessment of sensitizations, which was rarely (or never) tested in vitro and/or in vivo in the past. Thus, this feature allows the allergist to better define the IgE profile of the patient, and in certain cases, to improve the identification of the therapeutic strategy, particularly in food allergies. Along this line, it should be considered that the allergen and component selection made by the producers seems to be almost exhaustive. However, if some component, such as omega-5-gliadin, Tri a 14 and alpha-Gal, is inserted in the assay, the diagnostic power of this tool could be further improved.

Conclusion

In conclusion, ALEX, the immunoassay for specific IgE to whole allergens and relevant molecular components, is an interesting new approach to the bottom-up [10] diagnosis of allergies. The combination of extracts and components should save time and costs when an accurate allergy diagnosis is required, particularly considering AIT for polysensitized patients and patients with pollen/food syndromes. These features, together with the interesting results observed in the present study, show promise that this approach will capture the interest of allergists, particularly molecular allergists, in the near future, because of its direct impact on the management of patients with allergies in the context of a PM approach [1].

Abbreviations

AIT: Allergen Immunotherapy; ALEX: Allergy Explorer; AMA: Allergen Microarray; CCD: Cross-Reactive Carbohydrate Determinants; ISAC: Immuno-Solid-Phase Allergen Chip; MAD: Molecular Allergy Diagnosis; PM: Precision Medicine; SPT: Skin Prick Test

Acknowledgements

Dr. Christian Harwanegg, CEO of MacroArrayDx, Wien (Austria), shared with the Author the data of pre-clinical validation of the assay.

Funding

This work was partially supported by Associazione Ricerca Malattie Immunologiche e Allergiche (ARMIA), Genova, Italy. A technical support was obtained by MacroArrayDx, Wien (Austria).

Availability of data and materials

Raw data obtained from the readers of the assays are available if needed.

Authors' contributions

EH performed the statistical analysis, wrote and revised the article; SP, FP and MM revised the article and greatly contributed to the discussion of the results, starting from their experience in the field of molecular allergy applied to clinics; GM performed the tests, revised the statistical analysis, wrote and revised the article; GWC revised the article under the light of a precision medicine approach. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This was a laboratory study where two methods commercially available were used to describe the IgE profile in a group of sera collected for diagnostic reasons in allergic patients. Sera were identified by a unique laboratory code and were processed in an anonymous procedure. All patients were informed of the fact that their serum could be used in a comparison with another assay and gave a verbal consent.

Competing interests

The authors declare that they have no competing interests.

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Received: 1 March 2018 Accepted: 6 April 2018
Published online: 26 April 2018

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