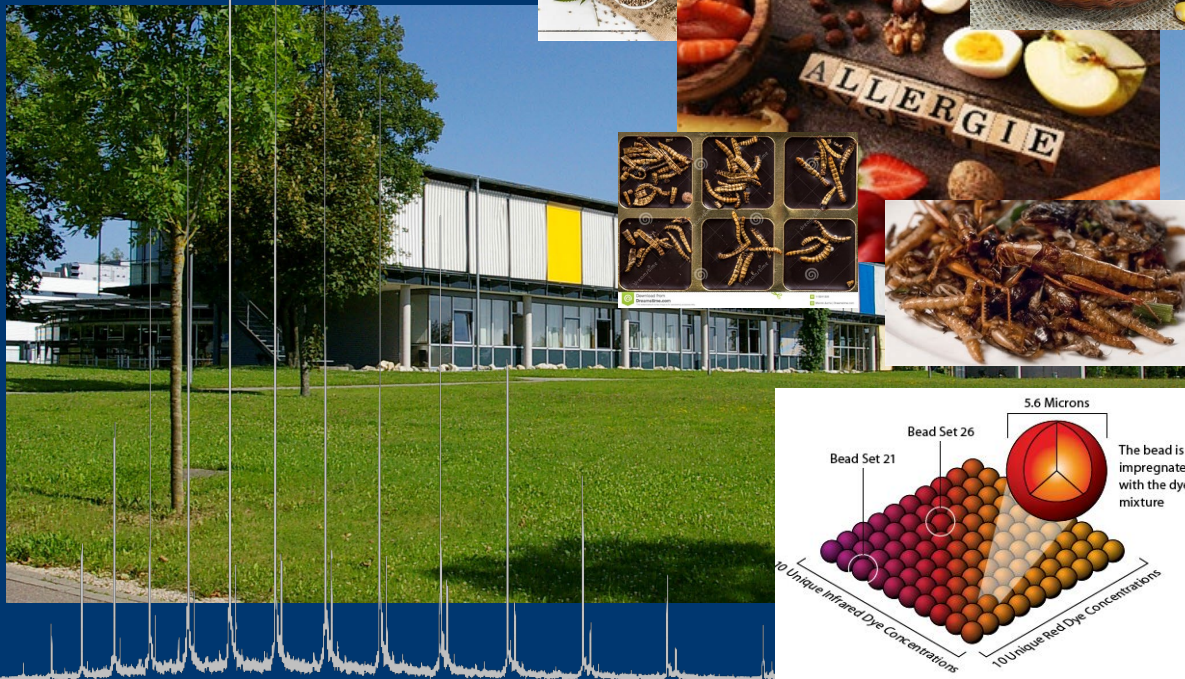


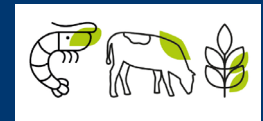
AllergenPro

Application of the DigiWest protocol on the allergens from insects, peanut, shrimps and HDM



23- nov-2023

BfR Akademie



Workshop Authentifizierung von allergenen Bestand-teilen, Tierarten und Geweben in Lebens- und Futtermitteln

Prof. Dr. Dieter Stoll
Albstadt-Sigmaringen
University of Applied Sciences

AllergenPro

With support from



by decision of the
German Bundestag

Project manager



Grant No: **281A304D18**

09/2020 – 02/2024

„Novel Food“ – Novel Health Risks ?!

"Novel Foods"



AllergenPro



- Products that were practically not consumed in the EU before 1997..
- regulated via EU regulation since 01/2018 – **insects are Novel Food = need EU approval**
- Stringend scientific assessment of food safety by European Food Safety Authority (EFSA) to recommend / deny approval & proposed (allergen) risk labelling
- 8 applications for insects as novel food (10/2023)



Risk if allergic to **insect venoms**?










Risk if allergic against **crustaceans**?



Risk if allergic to **house dust mites**?

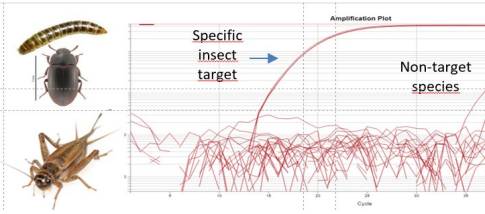


e.g. mealworm

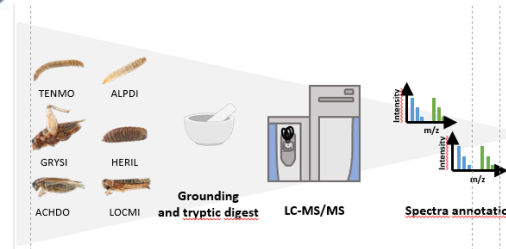
	Yellow / common Mealworm <i>Tenebrio molitor</i>	06/2021	✓
	Migratory Locust, grasshopper <i>Locusta migratoria</i>	11/2021	✓
	House Cricket <i>Acheta domesticus</i>	02/2022 01/2023	✓
	Lesser Mealworm <i>Alphitobius diaperinus</i>	01/2023	✓
	Black Soldier Fly (larvae) <i>Hermetia illucens</i>		??
	Tropical House Cricket <i>Gryllobates sigillatus</i>		??
	Two Spotted Cricket <i>Gryllus bimaculatus</i>		??
...			

Source: URL: https://food.ec.europa.eu/safety/novel-food/authorisations/approval-insect-novel-food_en
 URL: <https://www.bundesregierung.de/breg-de/aktuelles/insekten-in-nahrungsmitteln-2162992>

Allergen-Pro : Insect Food allergenicity assessment



Development of RT-PCR assays for detection of insect material in food.



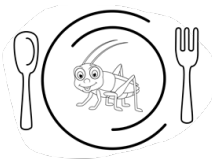
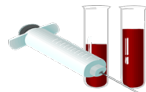
Development of IP-MS assays for quantification of insect proteins in food

With support from

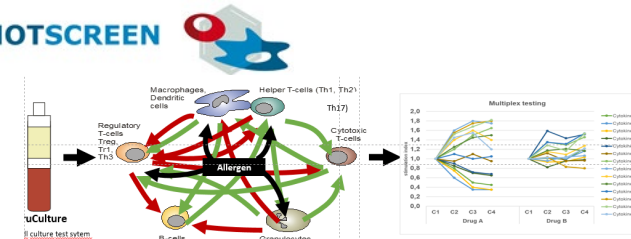


by decision of the German Bundestag

Project manager



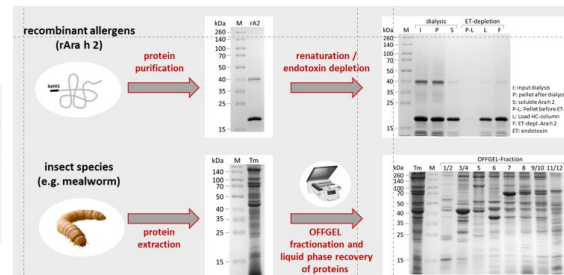
Patiententests – Analyse der allergenen Wirkung von Lebensmittelinsekten / Kreuzreaktivitäten



Development of a whole blood cell in-vitro system to measure allergic reactions with minimal patient risks

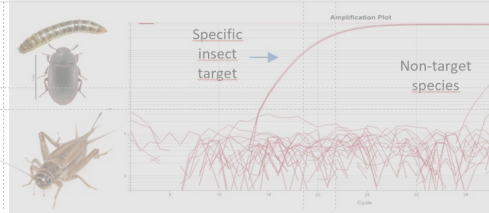
Grant No
 281A304A-F18
 09/2020-02-2024

(Rekombinante) Allergene für in-vitro Tests und IP-MS Methodenentwicklung
 Bestimmung der Allergenprofile von Novel Food Proteinen / WB & DigiWest

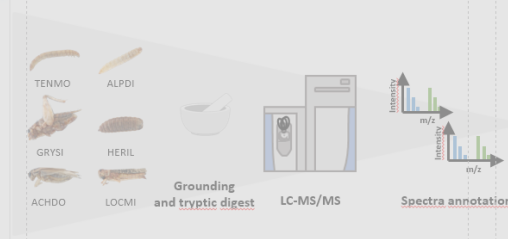


Institute of Food Safety and Analytical Sciences
 Lausanne, Schweiz

Allergen-Pro : Insect Food allergenicity assessment



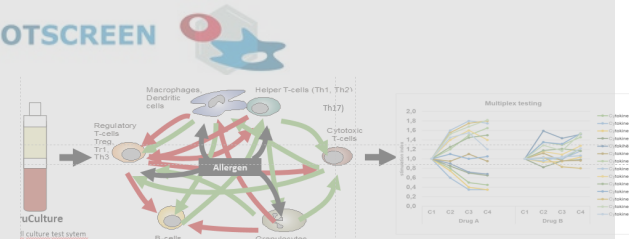
Development of RT-PCR assays for detection of insect material in food.



Development of IP-MS assays for quantification of insect test proteins in food



Patiententests – Analyse der allergenen Wirkung von Lebensmittelinsekten / Kreuzreaktivitäten



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With support from



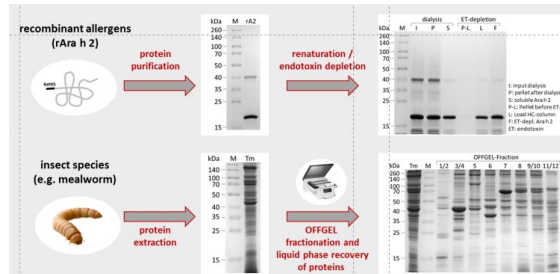
by decision of the German Bundestag

Project manager



Grant No
281A304A-F18
09/2020-02-2024

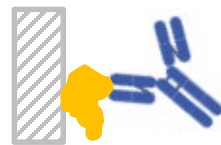
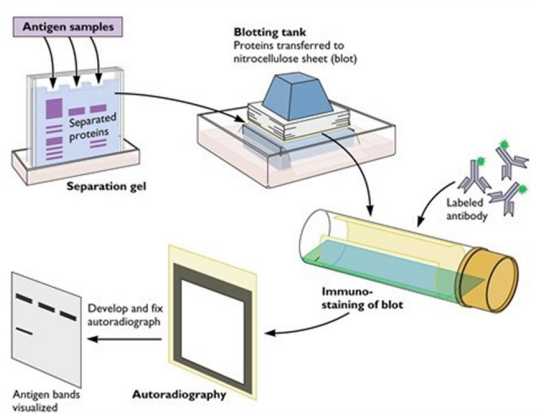
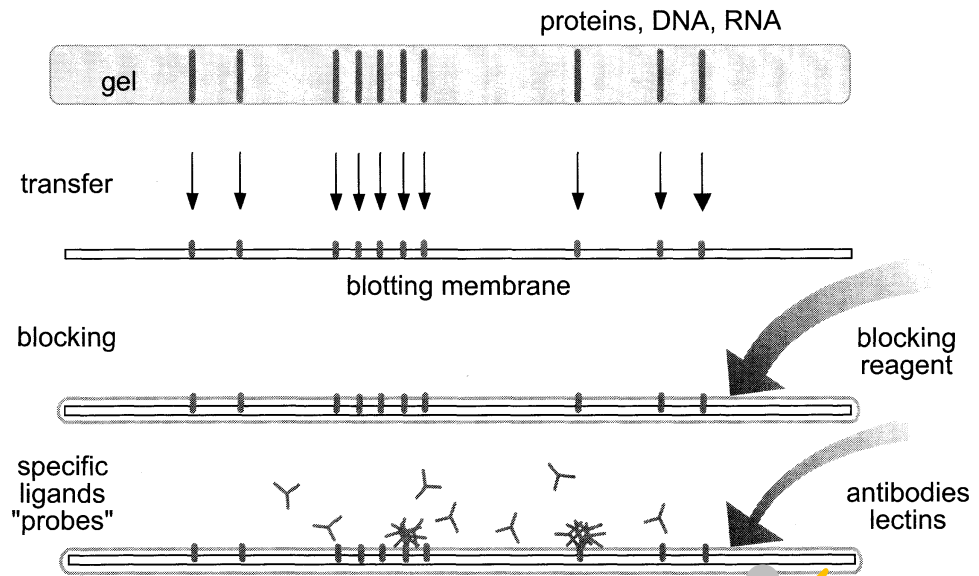
(Rekombinante) Allergene für in-vitro Tests und IP-MS Methodenentwicklung
Bestimmung der Allergenprofile von Novel Food Proteinen



Institute of Food Safety and Analytical Sciences
Lausanne, Schweiz

IgE profile –Immuno-Blot

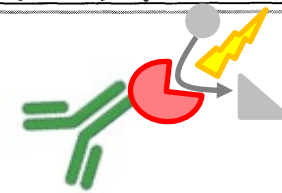
1. separation
(Elektrophoreses)
2. Transfer auf Membran
(„Blotting“)
3. Blocking of surface
4. Detection



A: IgE from sera of allergic patients



B: IgG specific for allergen

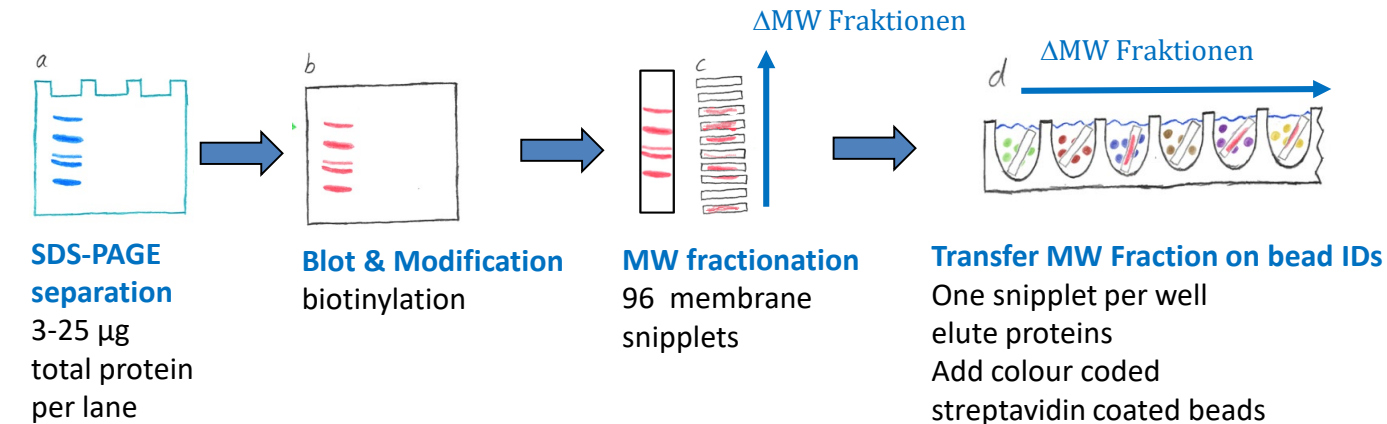


donkey anti-goat IgG - HRP
 donkey anti-goat IgG - HRP

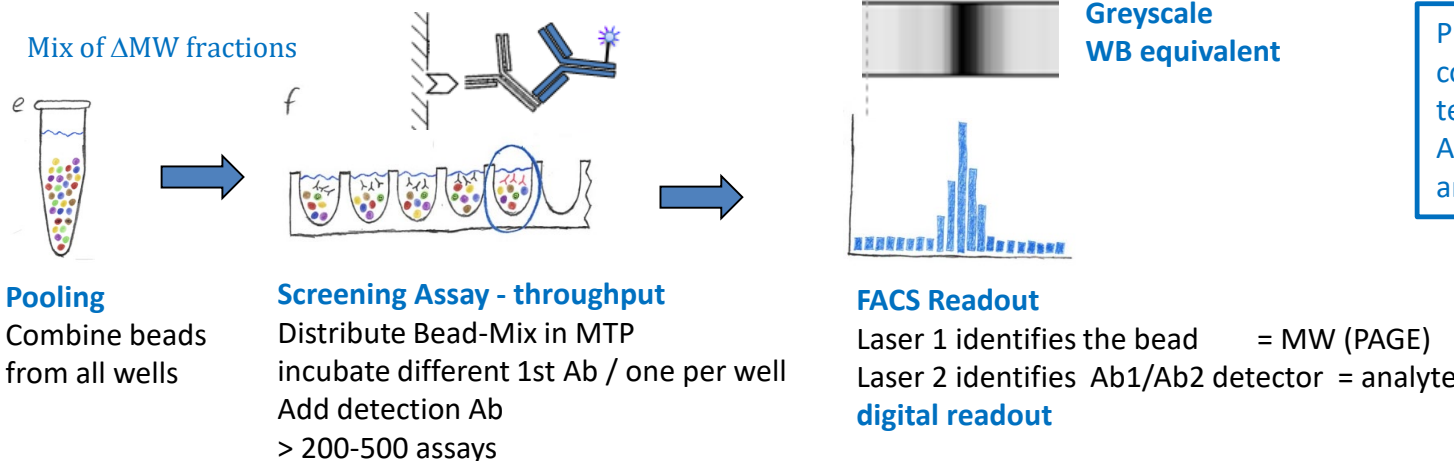
SuperSignal™ West Pico Plus
 (Luminol (Enhancer) H₂O₂)

DigiWest – Workflow

Markus Templin
Annette Döttinger

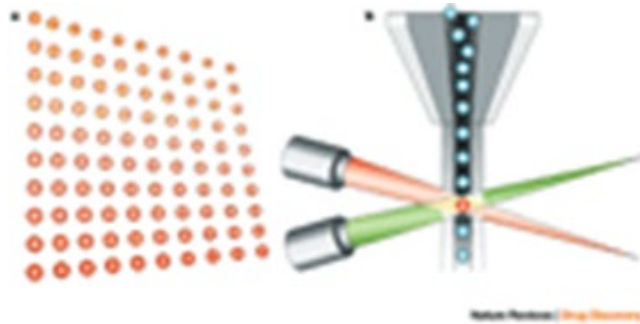


Protein MW fractions
are bound to
streptavidin on colour-
coded Luminex beads



Protein MW fractions on
colour coded beads are
tested with different prim
Abs binding to different
antigens

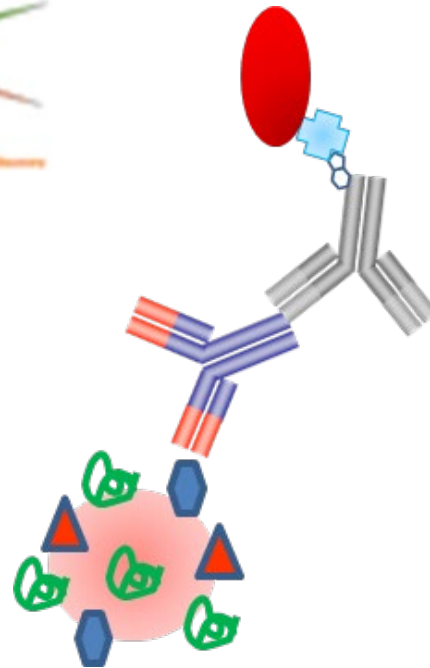
Luminex based Digi West readout



500 bead types
Work in MTP
FACS type bead identification
=> **bead colour (red laser)**

Analyte quantification by
fluorescence of phycoerythrin
green laser EX 545,569 nm
EM 580 yellow-orange

High detection sensitivity
Improved S/N ratio due to low
fluorescence background in
capillary



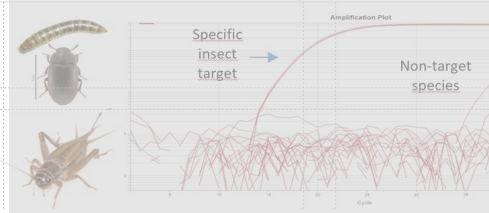
**Δ MW fraction of
Proteins on bead**

- 4th: **streptavidin** labelled with
phycoerythrin (fluorescent dye)
- 3rd: **species specific biotinylated
secondary antibody**
- 2nd: **Analyte specific primary antibody**
- 1st: **Colour coded bead**
+ all different biotinylated
proteins of a Δ MW fraction bound
to streptavidin surface

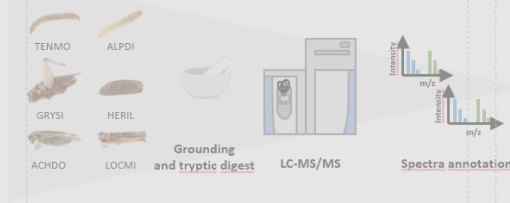


2. Food Allergen Profiles Immunoblots & DigiWest Allergen

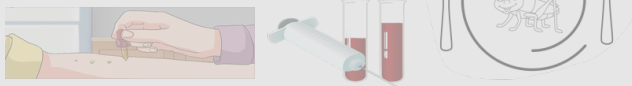
Allergen-Pro : Insect Food allergenicity assessment



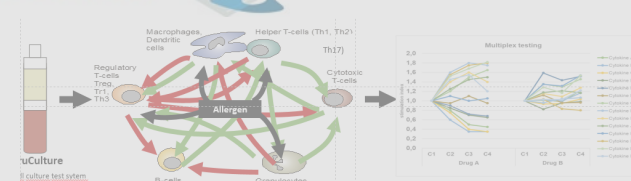
Development of RT-PCR assays for detection of insect material in food.



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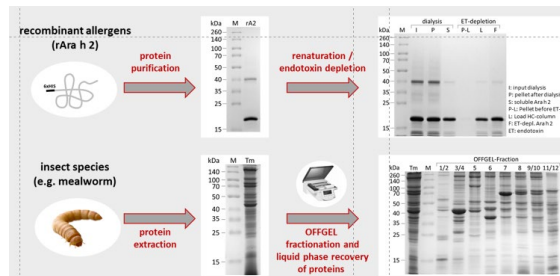
Patiententests – Analyse der allergenen Wirkung von Lebensmittelinsekten / Kreuzreaktivitäten



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and Analytical Sciences
Lausanne, Schweiz

Human Test Sera



CentralBiohub, Biospecimens Online [https://centralbiohub.de/]



2 x 

2 x 

4 x 

12 x 





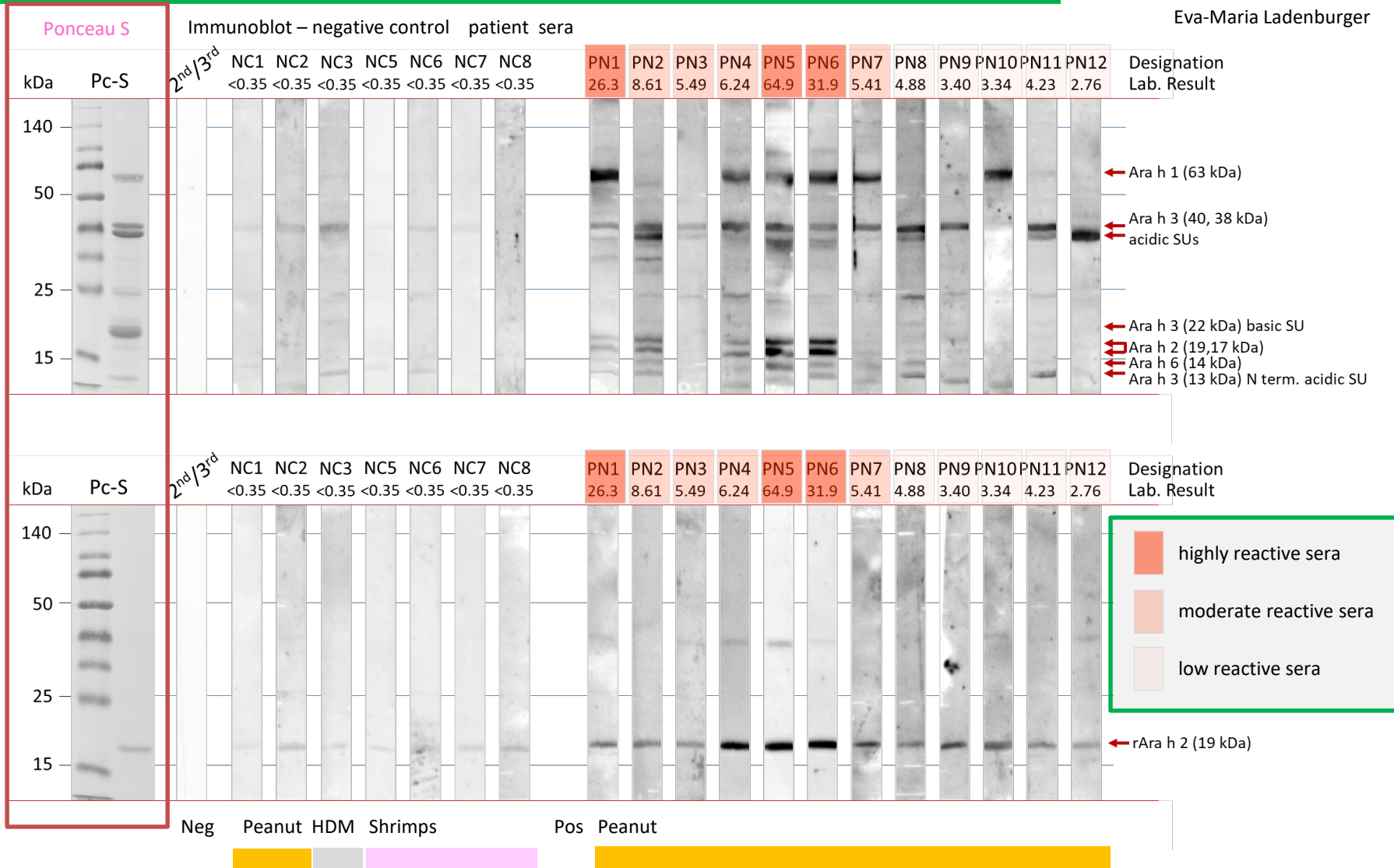
Int. Design. HSAS	Laboratory Lab_Parameter	Laboratory Result Interpretation	Laboratory Result_Raw	Laboratory Result_Numerical	further notes	Quantity Inform Quantity	Aliquots (50 µl) HSAS
Sera from Central BioHub							
NC1	sigE Peanut (f13)	negative	< 0.35	0.35	also neg. for HDM, Sr/Cr, ...pos for wasp	0.50	5x
NC2	sigE Peanut (f13)	negative	< 0.35	0.35	also negative for HDM, wasp, bee.....	1.00	10x
NC3	sigE Dermatophagoides pteronyssinus (d1)	negative	< 0.35	0.35	also negative for peanut, HDM, minor against wasp...	0.50	5x
NC4	sigE Dermatophagoides pteronyssinus (d1)	negative	< 0.35	0.35	also neg. for peanut..., ...only minor pos for dog dender	1.00	10x
NC5	sigE Shrimp (f24)	negative	< 0.35	0.35	also negative for peanut, HDM, wasp, bee.....	2.30	23x
NC6	sigE Shrimp (f24)	negative	< 0.35	0.35	also negative for peanut, HDM, wasp, bee.....	2.10	22x
NC7	sigE Shrimp (f24)	negative	< 0.35	0.35	also negative for peanut, HDM, wasp, bee.....	2.40	24x
NC8	sigE Shrimp (f24)	negative	< 0.35	0.35	atopic; highly reactive for hazelnut; neg. for peanut, HDM, shrimp	1.00	10x
PN1	sigE Peanut (f13)	positive	26.30	26.30		0.50	6x
PN2	sigE Peanut (f13)	positive	8.61	8.61	highly allergic to hazelnut	0.60	6x
PN3	sigE Peanut (f13)	positive	5.49	5.49		1.00	10x
PN4	sigE Peanut (f13)	positive	6.24	6.24		1.30	13x
PN5	sigE Peanut (f13)	positive	64.90	64.90	only tested for peanut	1.10	11x
PN6	sigE Peanut (f13)	positive	31.90	31.90	only tested for peanut	0.50	6x
PN7	sigE Peanut (f13)	positive	5.41	5.41	only tested for peanut	0.50	6x
PN8	sigE Peanut (f13)	positive	4.88	4.88	max. for cat dender	0.50	6x
PN9	sigE Peanut (f13)	positive	3.40	3.40			
PN10	sigE Peanut (f13)	positive	3.34	3.34			
PN11	sigE Peanut (f13)	positive	4.23	4.23			
PN12	sigE Peanut (f13)	positive	2.76	2.76			
HDM1	sigE Dermatophagoides pteronyssinus (d1)	positive	100.00	100.00			
HDM2	sigE Dermatophagoides pteronyssinus (d1)	positive	42.80	42.80			
HDM3	sigE Dermatophagoides pteronyssinus (d1)	positive	88.10	88.10			
HDM4	sigE Dermatophagoides pteronyssinus (d1)	positive	45.40	45.40			
HDM5	sigE Dermatophagoides pteronyssinus (d1)	positive	52.00	52.00			
CrSr1	sigE Crab (f23)	positive	11.40	11.40			
CrSr2	sigE Crab (f23)	positive	29.00	29.00			
CrSr3	sigE Crab (f23)	positive	13.40	13.40			
CrSr4	sigE Crab (f23)	positive	>= 100	100.00			
Cr1	sigE Lobster (f80)	positive	2.51	2.51			
Cr2	sigE Crab (f23)	positive	7.50	7.50			
Cr3	sigE Crab (f23)	positive	7.50	7.50			
Cr4	sigE Crab (f23)	positive	7.50	7.50			
Cr5	sigE Crab (f23)	positive	12.40	12.40			
Sr1	sigE Shrimp (f24)	positive	2.70	2.70			
Sr2	sigE Shrimp (f24)	positive	2.97	2.97			
Sr3	sigE Shrimp (f24)	positive	3.10	3.10			
HDM: House dust mite / PN: Peanut / HE: Hen egg / ST: Shrimp							
Internal donor sera							
A*	internal sample; penicillin allergy; no reaction to house dust mites; no food allergy known						
Lm*	internal sample; suspected allergy to house dust mites; skin reaction/urticaria after eating locusts						



sample-ID	Allergy	Sensitization	Insect Prick Test positive
AP-SR-010	HSM	HSM, PN	YES mealworm (TM)
AP-SR-005	PN	HSM	
AP-SR-017	HSM, HE	PN	
AP-SR-011	HSM		YES buffalo worm (AT)
AP-SR-012	ST		
AP-SR-013	PN		
AP-SR-014	PN	HSM	
AP-SR-015			YES buffalo worm (AT)
AP-SR-016	HSM, PN		

Reference Standard – Peanut

Eva-Maria Ladenburger



Crossreactivity Peanut – Lupine

Eva-Maria Ladenburger

Lupinensamen
L-S



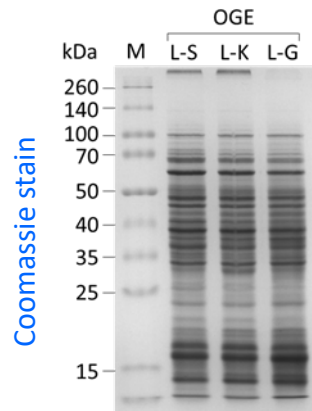
Lupinenkerne
L-K



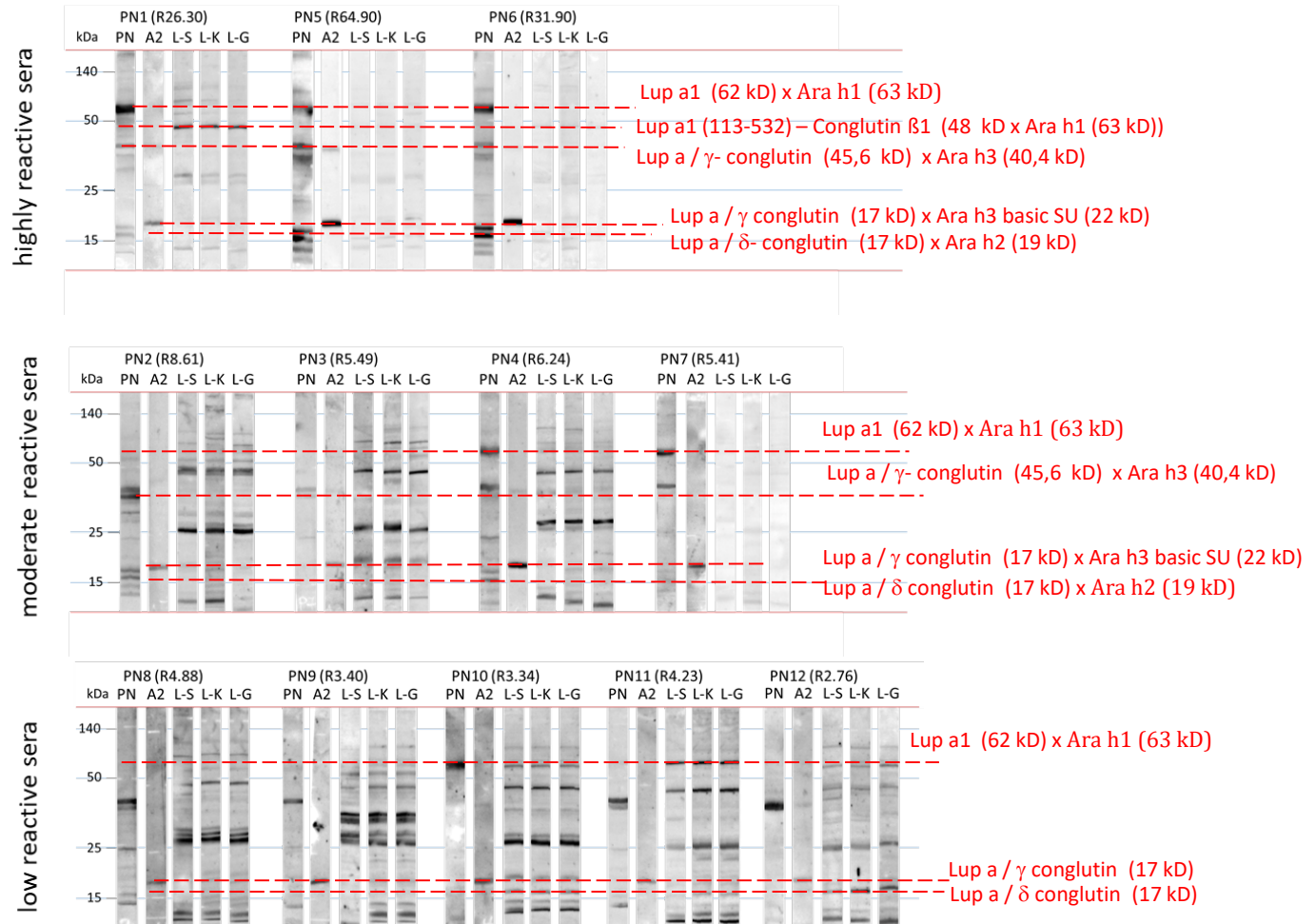
Lupinen gekeimt
L-G



Sera of peanut allergic patients (PN 1-12)

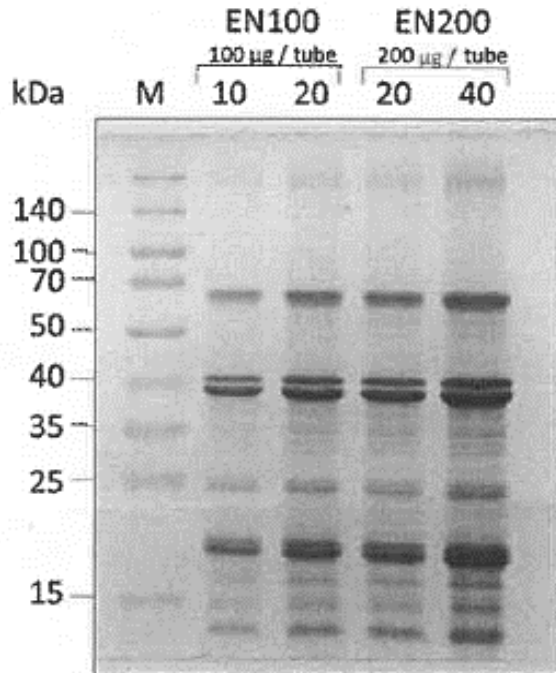


Protein IDs (UniProt / Proteom UP000464885)	Klassifizierung	Allergene (Lupinus albus)	Kreuzreaktionen mit Allergenen aus Leguminosen	
			Erdnuss	Soja
Q53154	α-Conglutin Legumin-like protein, 11S globulin fraction, ααα	Lup-2	Ara h 3	Gly m 6
Q53HY0; ADA6ASAZ23; ADA6ASMT4	β-Conglutin Vigilin-like protein; 7S globulin fraction; βββ	Lup-1	Ara h 1	Gly m 5
ADA6ASMD06; ADA6ASPA12; Q5F5H9	γ-Conglutin Vigilin-like protein; 7S globulin fraction	Lup a γ-conglutin	Ara h 3	n.d.
Q333K7; ADA6ASLHE1; ADA6ASLQ32; ADA6ASLQ02	δ-Conglutin Prolamin; 2S albumin superfamily	Lup a δ-conglutin	Ara h 2 Ara h 6 Ara h 7	Gly m 8
ADA6ASMN90; ADA6ASNVL3; ADA6AAQ2W5; ADA6AAPQ03; ADA6ASL5Y7; ADA6ASNT24; ADA6ASMPJ4	Profilin Profilin; Actin-binding proteins	Lup a 5	Ara h 5	Gly m 3
ADA6A4PB9	PR-10 proteins Bet v 1 type allergen; putative START-like domain	Lup a 4	Ara h 8	Gly m 4

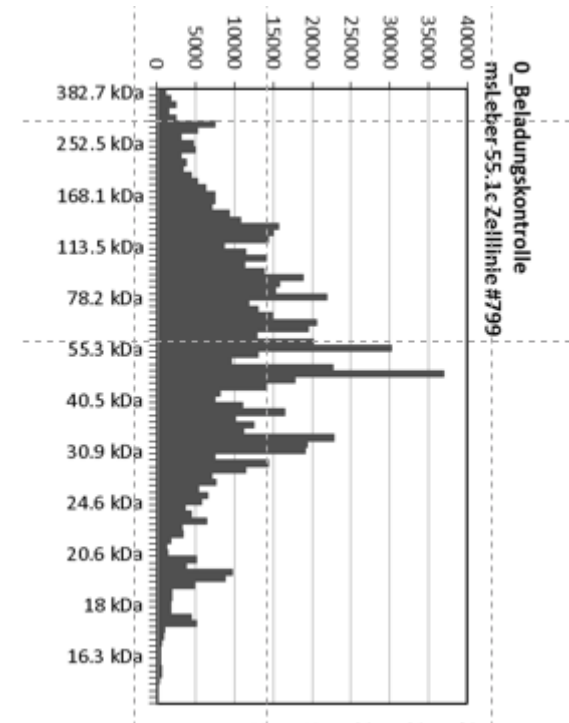
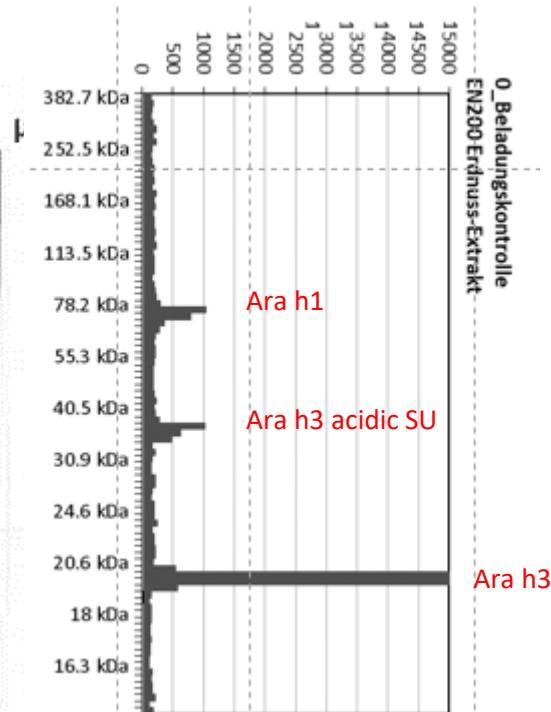


Peanut samples – DigiWest – Allergen Bead loading equivalence to PAGE

Peanut samples – PAGE
Coomassie Stain



DigiWest – Loading - control
Signal Histogram



Streptavidin-Phycoerythin (Strep-PE)
Binds to all biotinylated proteins on the bead

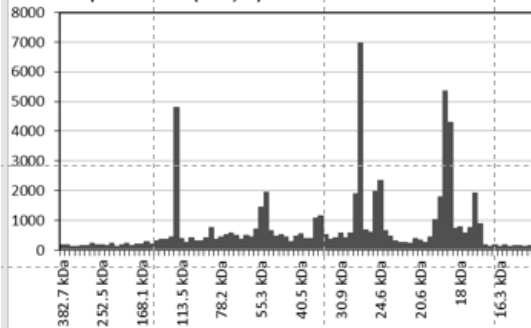


Mous liver lysate

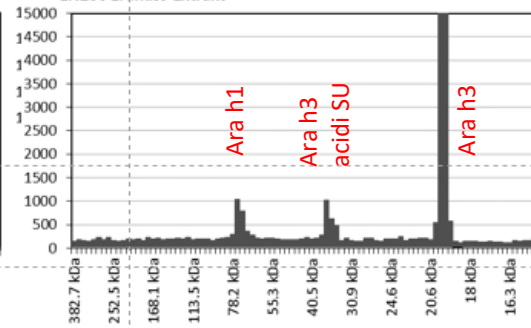
DigiWest – Peanut & Lupine



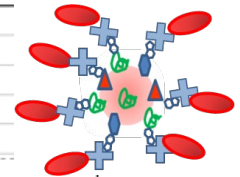
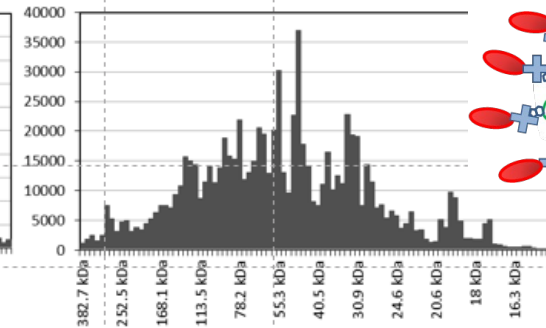
0_Beladungskontrolle
L-S Lupinen-Samen (WCE, fd)



0_Beladungskontrolle
EN200 Erdnuss-Extrakt

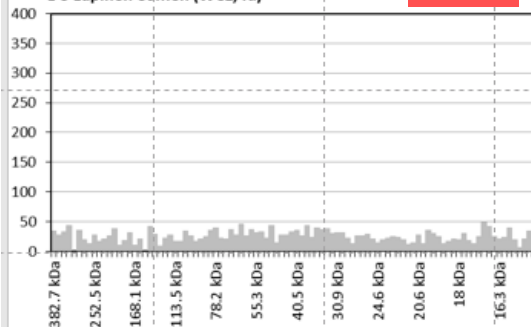


0_Beladungskontrolle
msLeber 55.1c Zelllinie #799

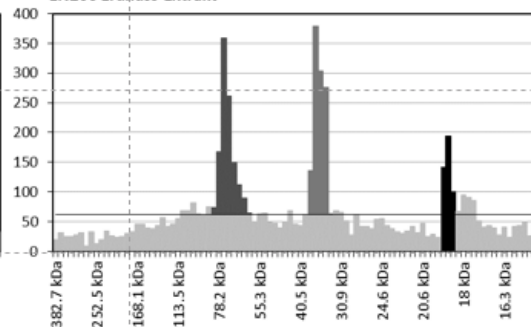


5_PN5 - Erdnuss 1:25
L-S Lupinen-Samen (WCE, fd)

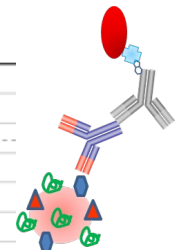
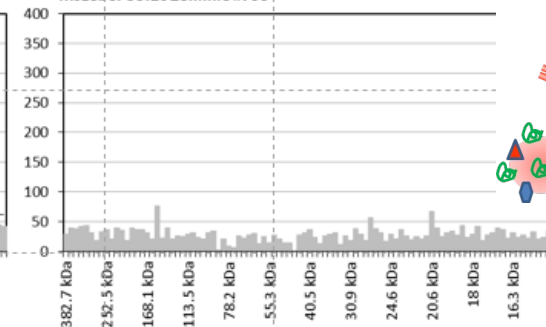
PN 5



5_PN5 - Erdnuss 1:25
EN200 Erdnuss-Extrakt

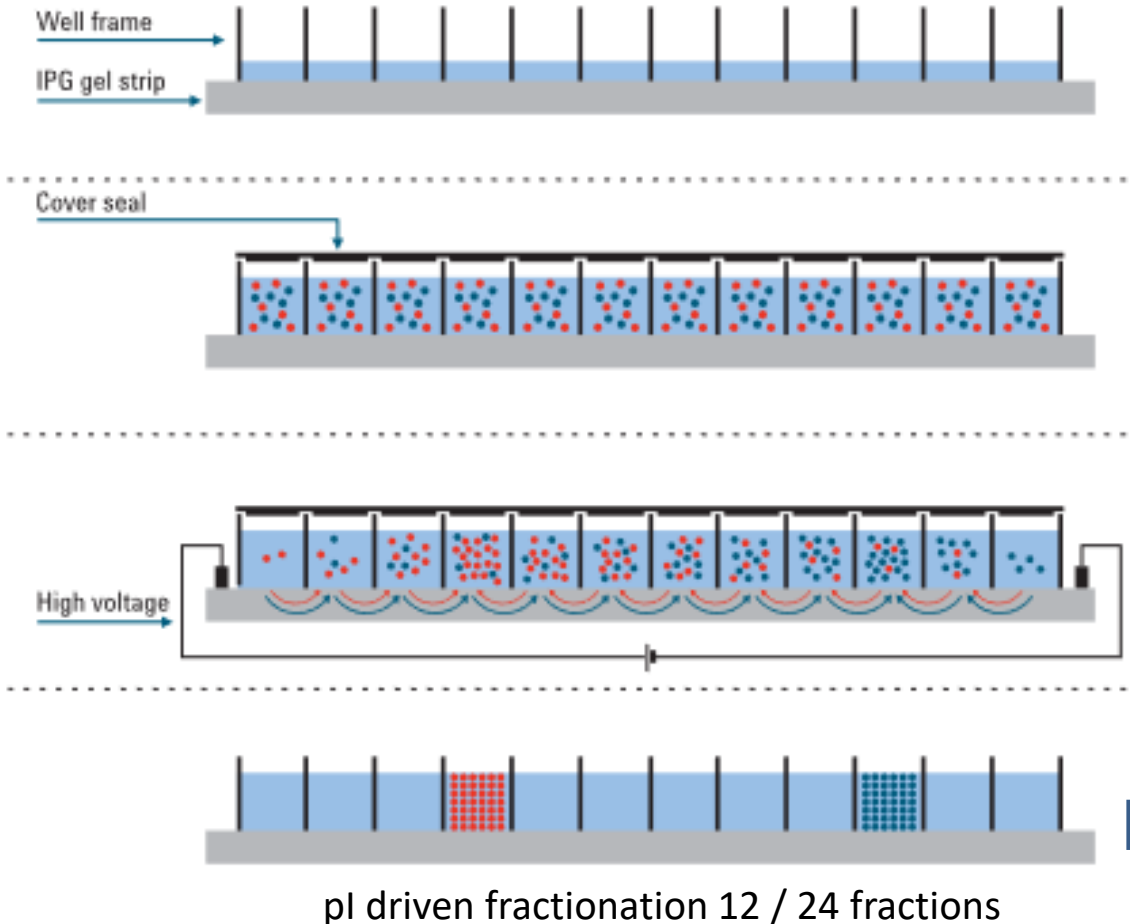


5_PN5 - Erdnuss 1:25
msLeber 55.1c Zelllinie #799



OFFGEL Fractionation of extracted proteins – Optimized Identification of allergenic proteins

Agilent 3100 OFFGEL Fractionator

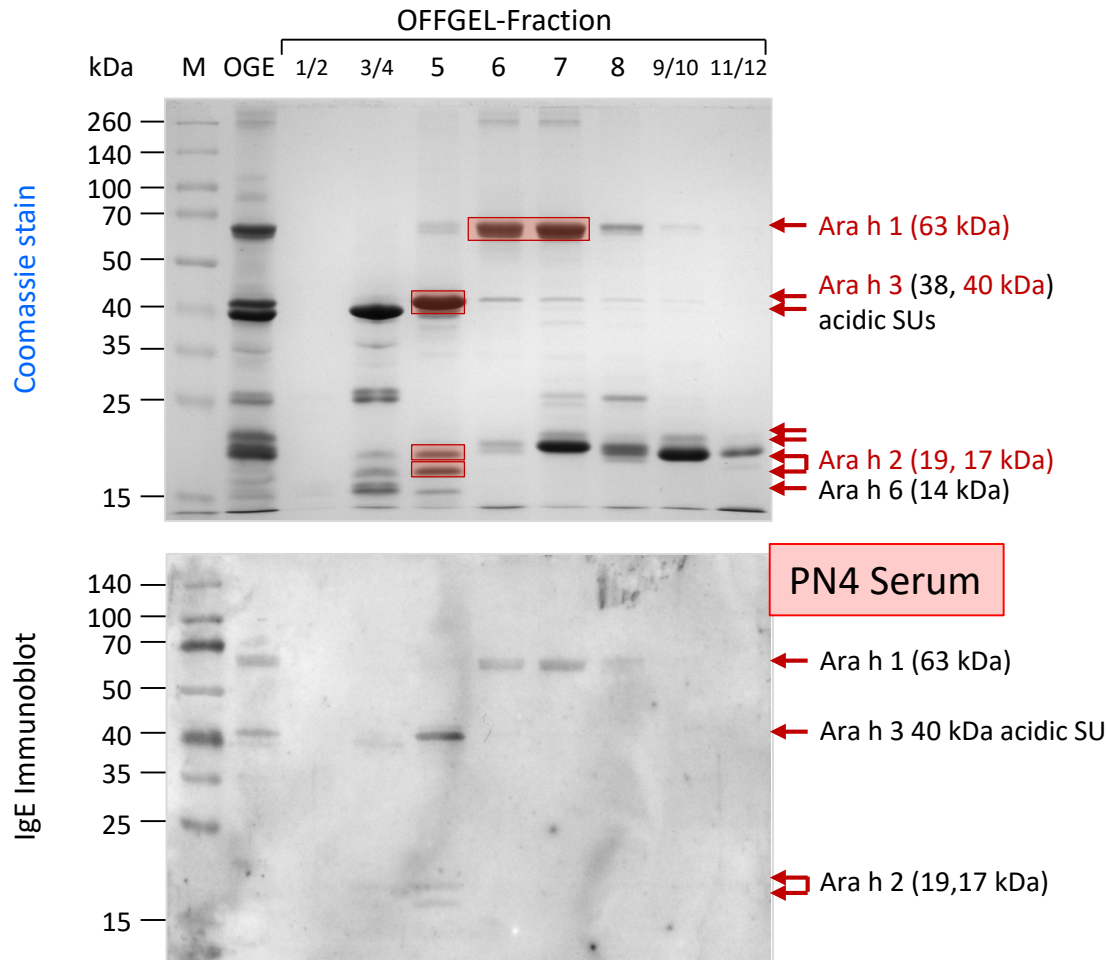


SDS-PAGE
Shotgun MSMS ID
DigiWest

OFFGEL Fractionation – characterization peanut IEF fractions



Eva-Maria Ladenburger



IgE immunoblot of PN4 results in signals of approx. 20 kDa, 40 kDa and 60 kDa in OFFGEL fractions 5 to 7

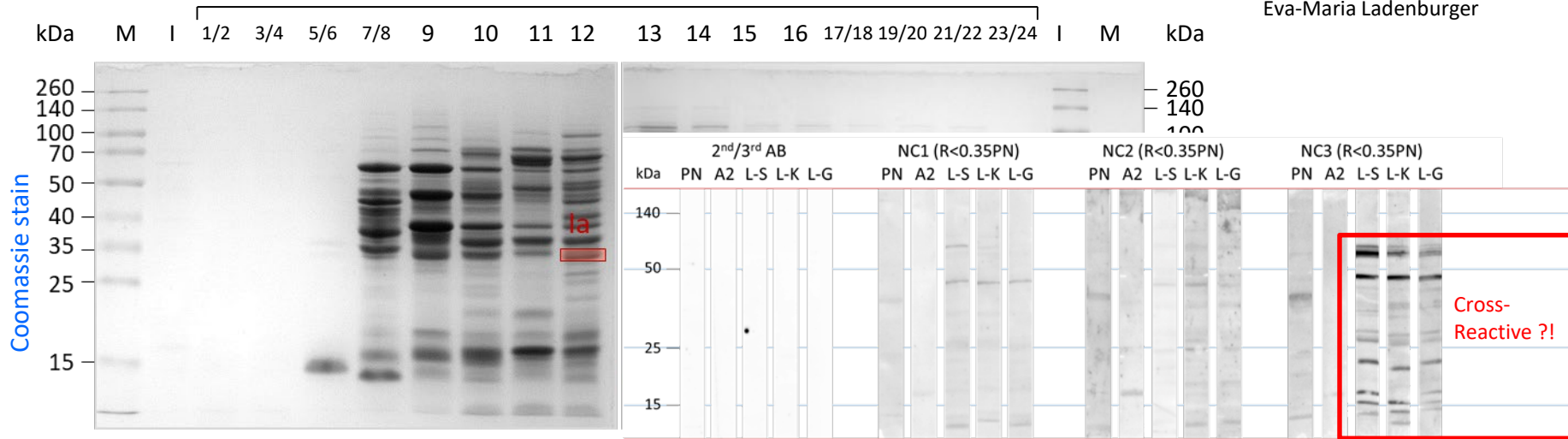
- The signals appear to be consistent with Ara h 1,
- 40 kDa acidic subunit of Ara h 3
- both isoforms of Ara h 2

Crossreactivity Peanut – Lupine

Lupine OFFGEL Fractions



Eva-Maria Ladenburger



MW	Allergens		ProtParam Expasy		
	Allergome	Lupinus Albus	pI (calc)	MW	
140	1	Lup an 3 3.0101	Non-specific lipid-transfer protein	9,35	9235,69
100	2	Lup an 3	Non-specific lipid-transfer protein	8,87	10200,84
70	3	Lup a4	Blad	9,66	20404,85
50	4	Lup a5	γ -Conglutin 2 alpha subunit	8,58	29034,05
40	1	Lup a1	Gamma conglutin 1 alpha subunit	8,73	29209,13
35	2	Lup a5	γ -Conglutin 1 alpha subunit	8,73	29209,13
25	3	Lup a1	Gamma conglutin 1	8,24	45608,68
25	4	Lup a5	γ -Conglutin 1	8,24	45608,68
15	5	Lup a5	γ -Conglutin 2	6,52	45873,89
15	6	Lup a alpha	Legumin-like protein	5,47	55718,76
15	7	Lup a4	Conglutin β 2	5,95	58894,65

Sample Preparation Process– Insect Extracts

1 – Select best raw material by total protein yield & protein distribution over the full MW range

mealworm



freeze dried



heat dried



frozen



2 – Select best milling - by total protein yield & protein distribution over the full MW range

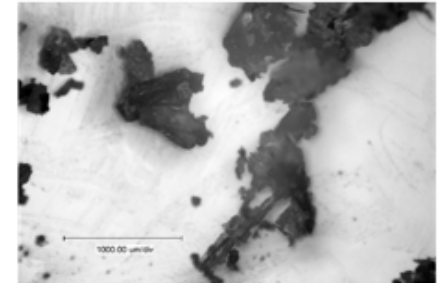
IKA ULTRA-TURRAX®
Tube Drive "ball mill"



IKA Tube Mill control
"tube mill"



T.m. dried (SFTE) – tube mill



3 – Select best extraction - by total protein yield & protein distribution over the full MW range

Soft extraction: according to Heick et al, 2011, J.Chromatogr. A 1218:938-943
Homogenized sample 3 h at 60°C in 50 mM TrisHCl pH 8.2

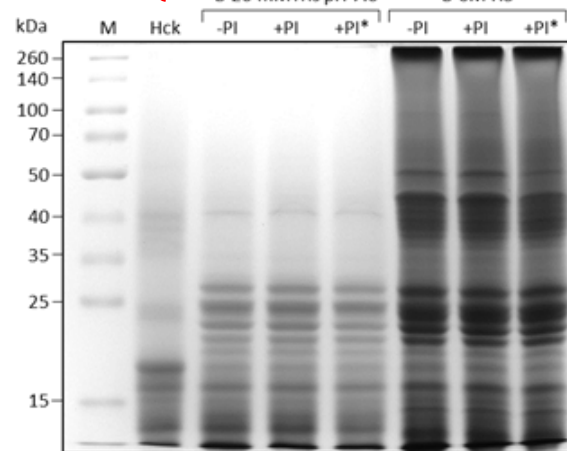
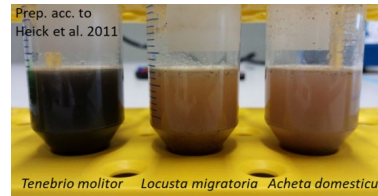
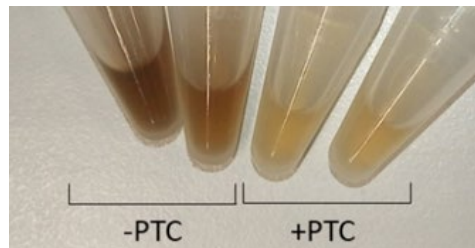
*Established for MS based allergen
quantificatio in food*

Soft extraction: according to Broekman et al, 2015, Mol.Nutr.Food Res. 59:1855-1864
Homogenized sample – 3-step – protein extraction - sequential treatment
A: low salt buffer (20 mM TrisHCl pH 7.6)
B: high salt buffer (6M urea in 20 mM TrisHCL pH 7.6)
C: denaturing SDS /DTT step

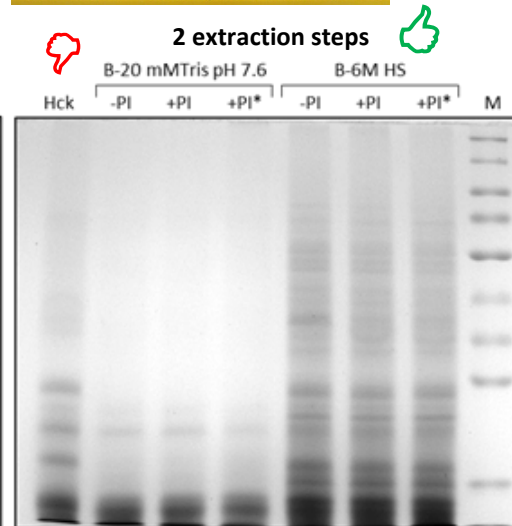
Sample Preparation – Insect Extracts

Comparison of published allergen extraction methods

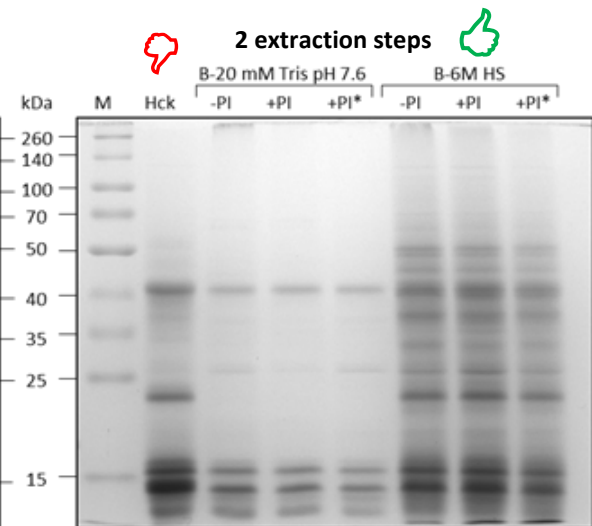
Eva-Maria Ladenburger



Tenebrio molitor [SFTE]



Locusta migratoria [SFTE]



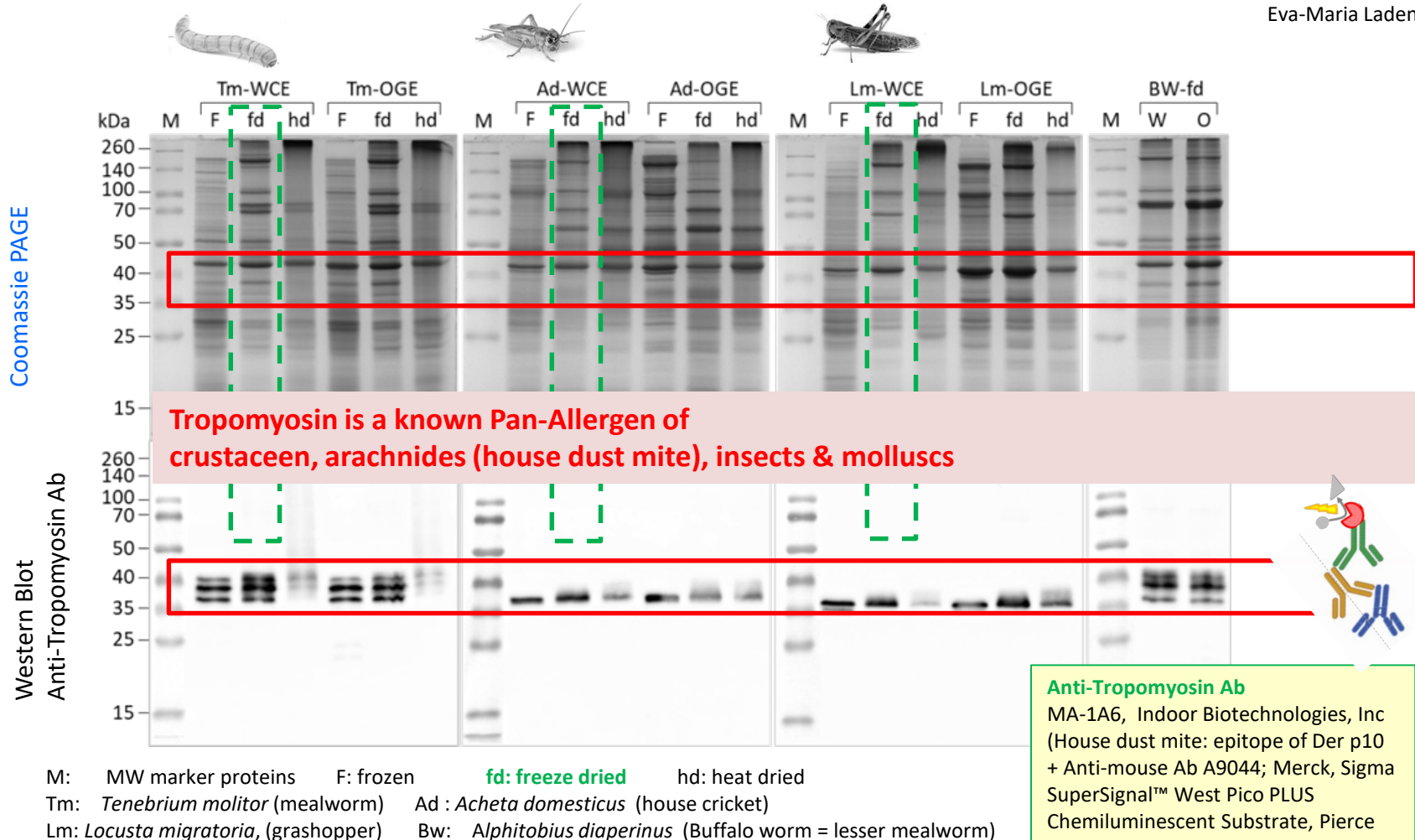
Acheta domesticus [SFTE]

M: MW marker proteins
 Hck: Extracts Heick et al, process
 B : Broekman et al, process
 -Tris: A step - low salt extract
 -6 M urea: B step – high salt extract
 +/- PI : with / without protease inhibitor
 (HALT inhibitor cocktail, Thermo)

Reproducible – but

- differences due to fat content and raw material processing
- Strong oxidation in low salt frozen insect extracts needs phenylthiocarbamate (PTC)

Sample Preparation – Insect Extracts final method



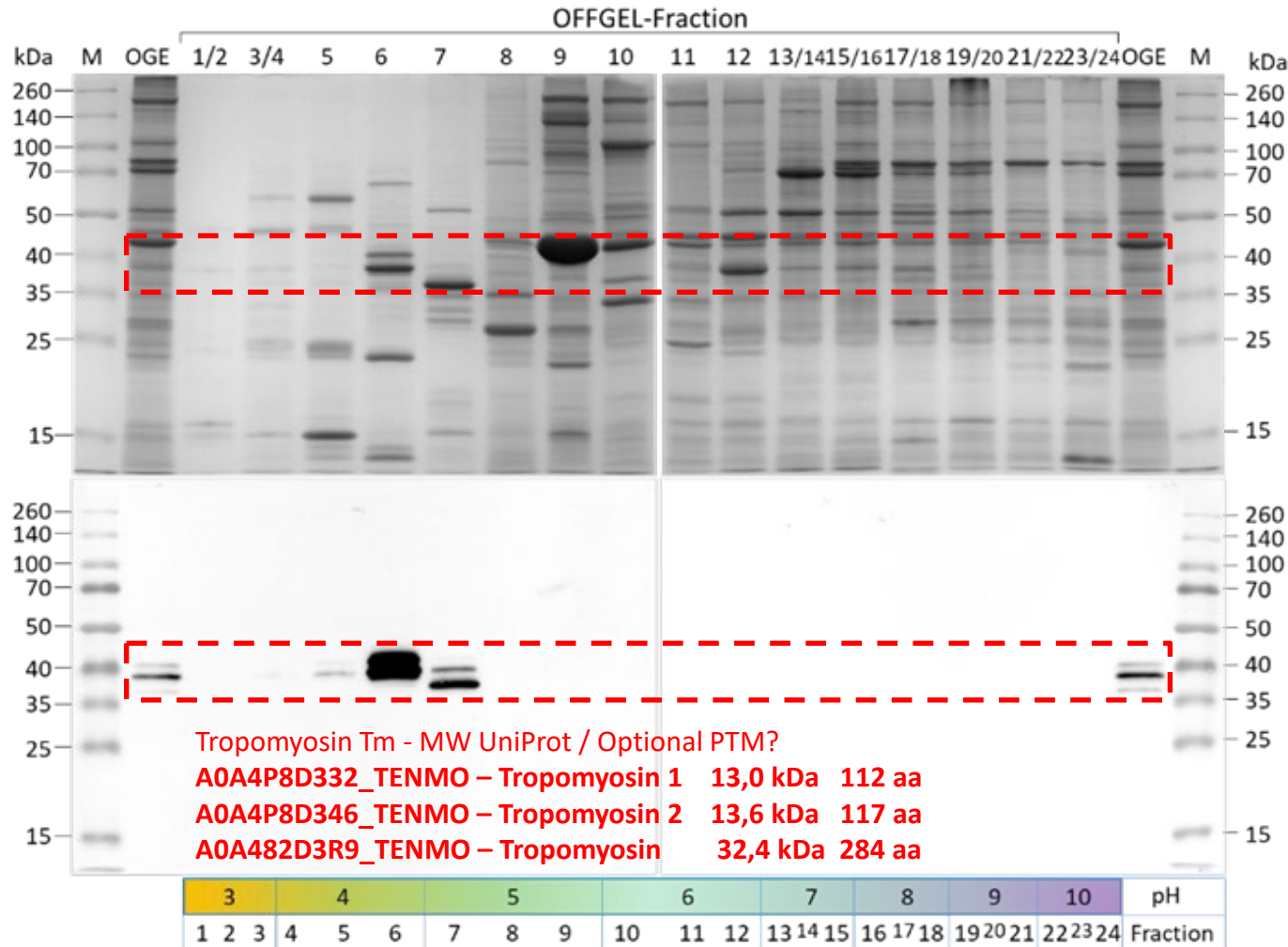
WCE / W: Whole Cell Extraction Protocol : 2,5 % SDS, 1 % DTT, 40 mM TrisHCl pH 8, 95°C, 10 min

OGE / O: OFFGEL Extraction Protocol : 1,25 x OFFGEL-buffer (8,75 M urea, 2,5 M Thiourea, 1,25 % DTT, 6,25 % Glycerol), 30°C, 90 min

OFFGEL Fractionation of extracted proteins – Identification of allergenic proteins



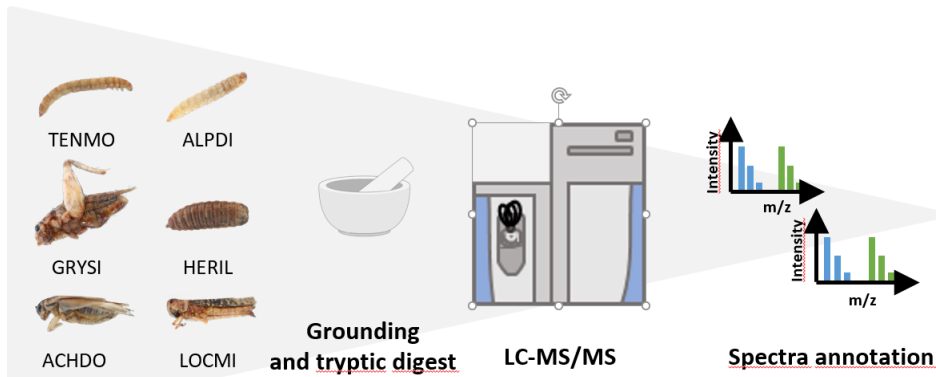
Mealworm, freeze dried (fd), OGE protocol



Anti-Tropomyosin Ab
 MA-1A6, Indoor
 Biotechnologies, Inc
 (House dust mite:
 epitope of Der p10
 + Anti-mouse Ab A9044;
 Merck, Sigma
 SuperSignal™ West Pico
 PLUS Chemi -
 luminescent Substrate,
 Pierce

- Allergen enriched fractions
- Improved allergen ID (Shotgun MSMS)

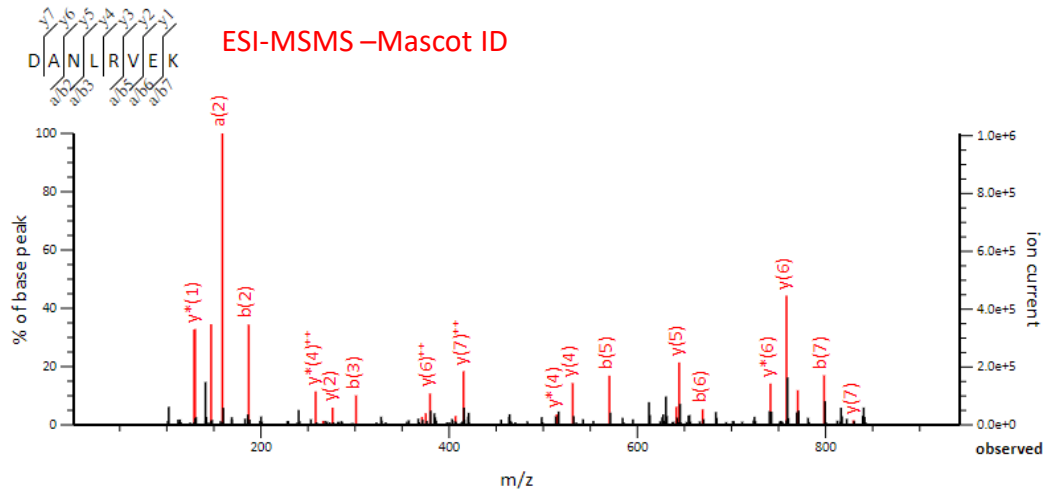
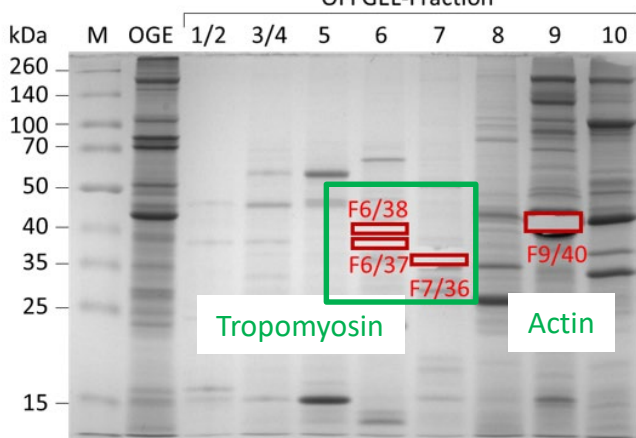
Shotgun MS based protein identification



Species	UniProtKB # entries (NOV-2021)	# proteins found in this study
<i>T. molitor</i>	634	1,150
<i>A. diaperinus</i>	47	440
<i>G. sigillatus</i>	37	360
<i>H. illucens</i>	17,599	2,051
<i>A. domesticus</i>	159	450
<i>L. migratoria</i>	1,559	600

Fully annotated protein databases are missing!
Use MS data to find insect proteins & species specific peptides

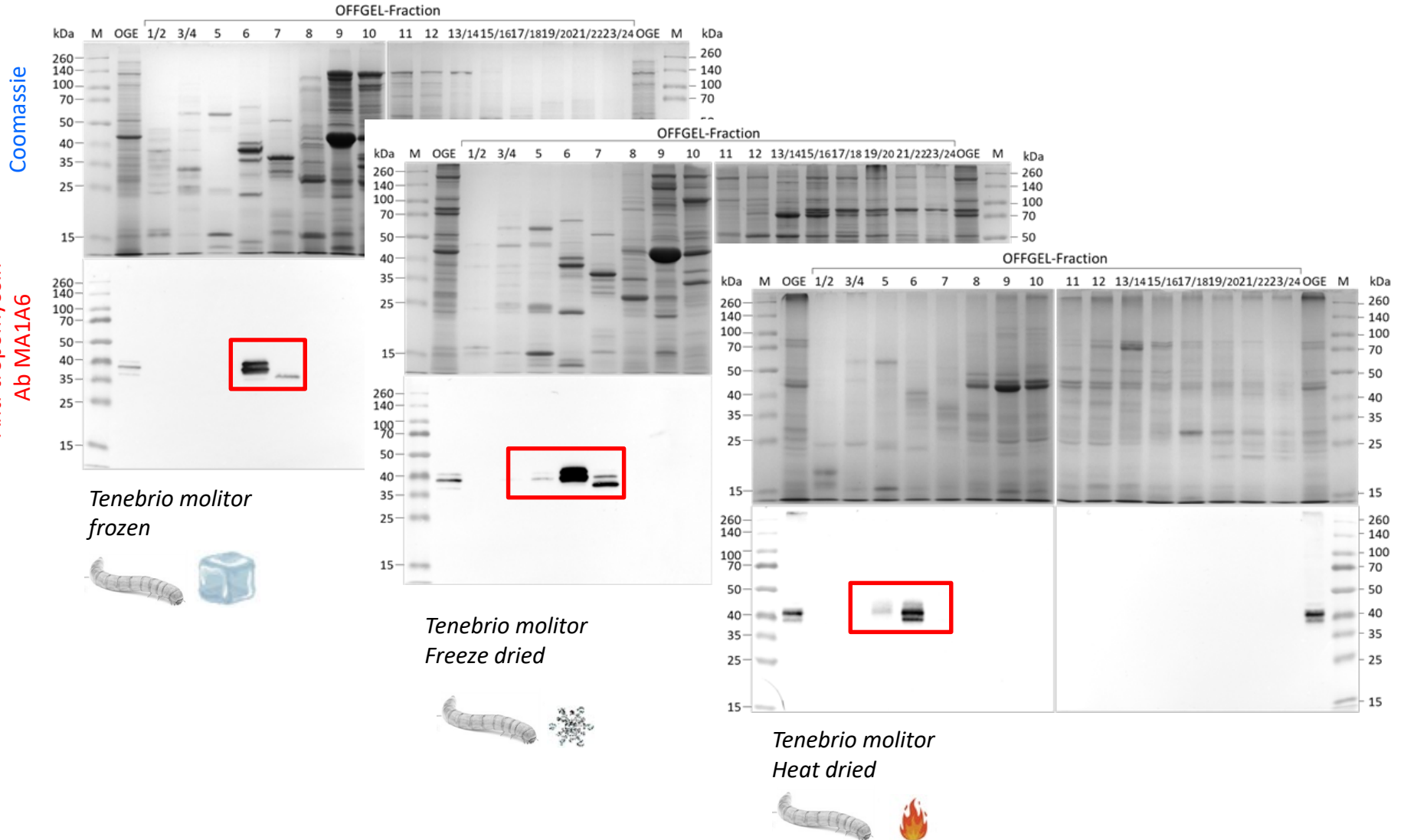
Fractions fd Tm



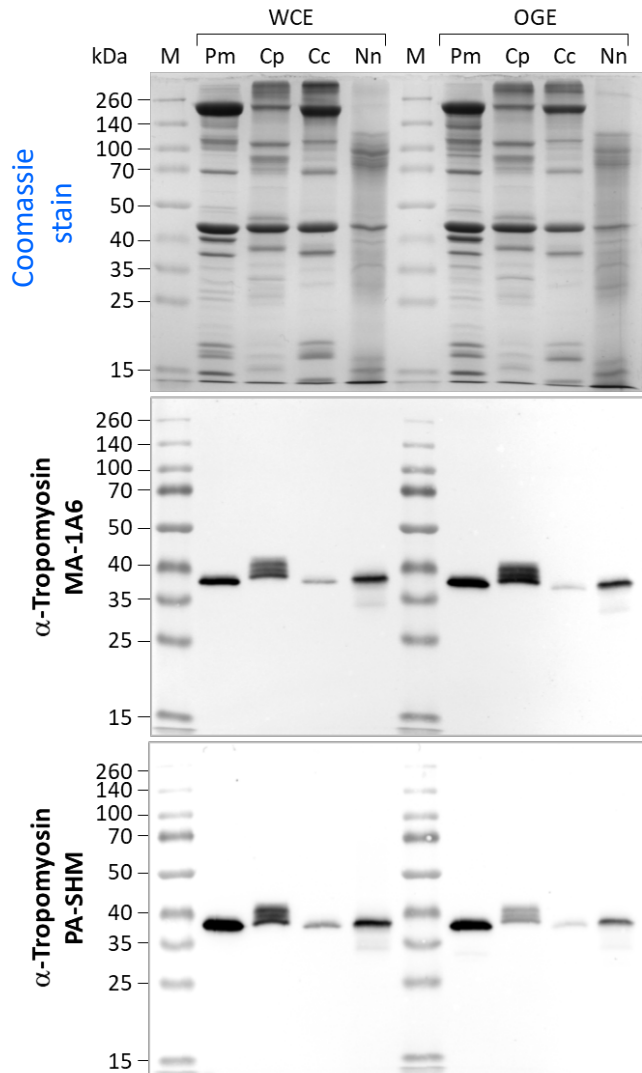
Identify allergen modifications caused by food processing



Mealworm – OFFGEL Fractions



Identify allergen in 4 crustacean species via IgE Immunoblot

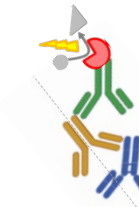
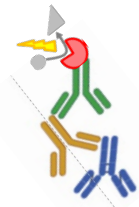


Pm: *Penaeus monodon* (Black Tiger Prawn)
 Cp: *Cancer pagurus* (crab);
 Cc: *Crangon crangon* (shrimp; North Sea shrimp);
 Nn: *Nephrops norvegicus* (Norway lobster; Scampi)

Two anti-Tropomyosin ab MA-1A6 and PA-SHM

Pm: strong - very strong 1 band
 Cp: medium - medium 2 bands
 Cc: weak - very weak 1 band
 Nn: strong - medium 1 band

Similar extractability in both protocols



Screening for IgE profiles in Novel Food sera of crab allergic patients

BtP: Black Tiger Prawn
Cp: crab (*Cancer pangurus*)
Cc: shrimp (*Crangon crangon*)
Nn: Norway lobster (*Nephros norvegicus*)

CrS4 crab positive serum
 Lab value: 100
 Cross reactivity highly reactive
 cockroach, HDM, wasp;
 peanut neg.

NC5 Negative control

Cr4 crab Positive serum
 Lab value: 100
 Cross reactivity Bee, cockroach,

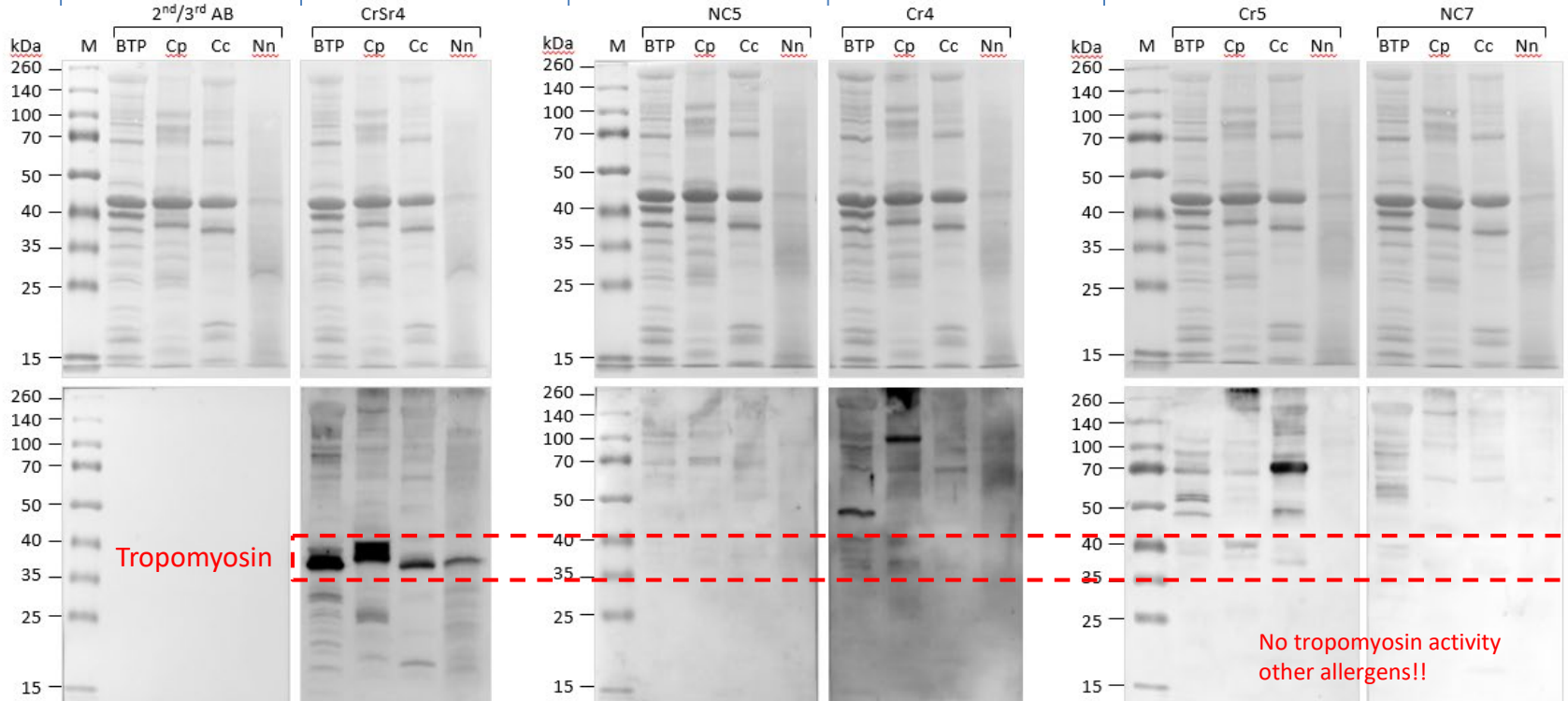
Cr5 crab Positive serum
 Lab value: 100
 Cross reactivity Pollen, shrimp

HDM negative

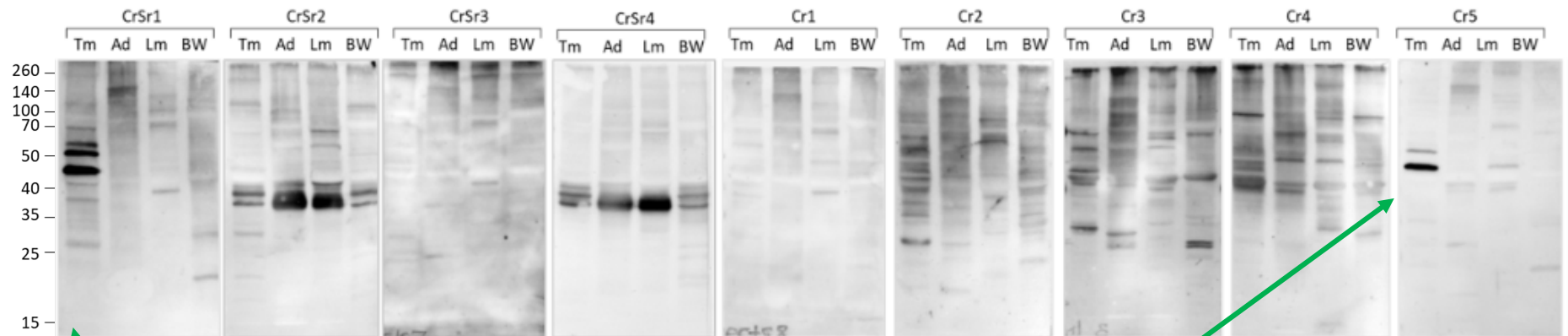
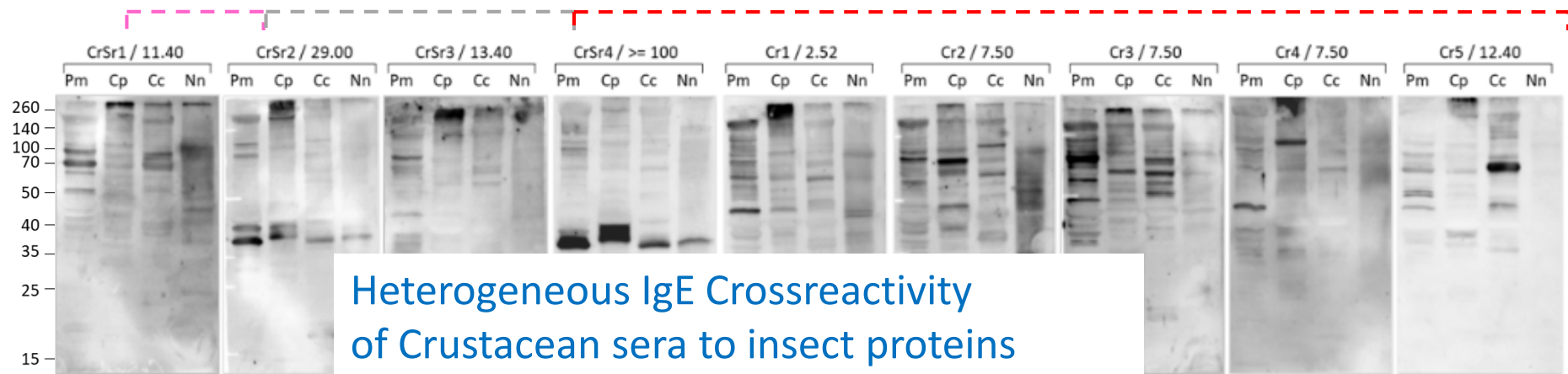
Ab control

Ponceau S
(Aufnahme 07.09.23)


 IgE Immunoblot
 (Detektion 11.09.23)



IgE immunoblot Screening of sera reactive to crustaceans - insect allergens

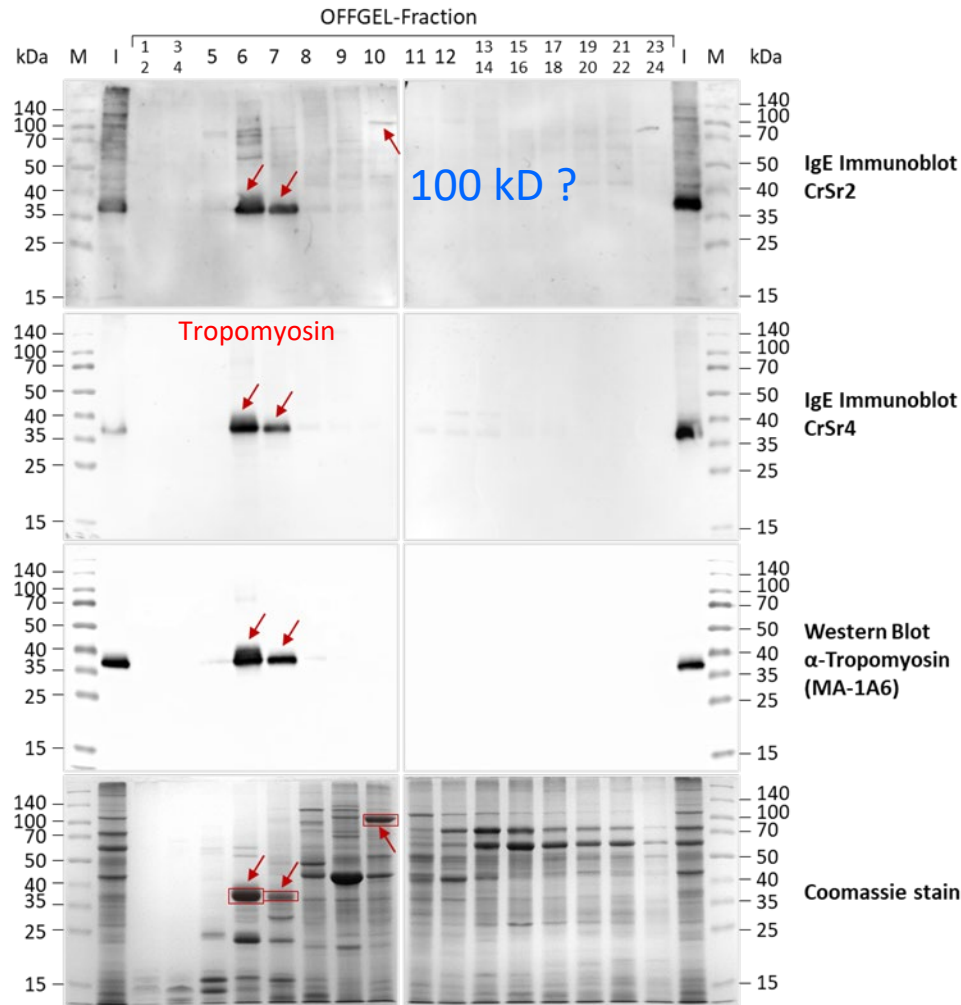


CrSr2 and CrSr4 IgE bind to insect tropomyosin?

signals obtained by CrSr1 and Cr5 are restricted to *Tenebrio molitor*?

Other sera IgE bind to other insect proteins

Crustacean IgE are crossreactive to IEF fractions of insect proteins



IgE immunoblot of CrSr2 and CrSr4
 sera from **crustacean allergic patients**
 tested on OFFGEL fractions of extracts from
House Cricket *Acheta domestica* (freeze-
 dried)

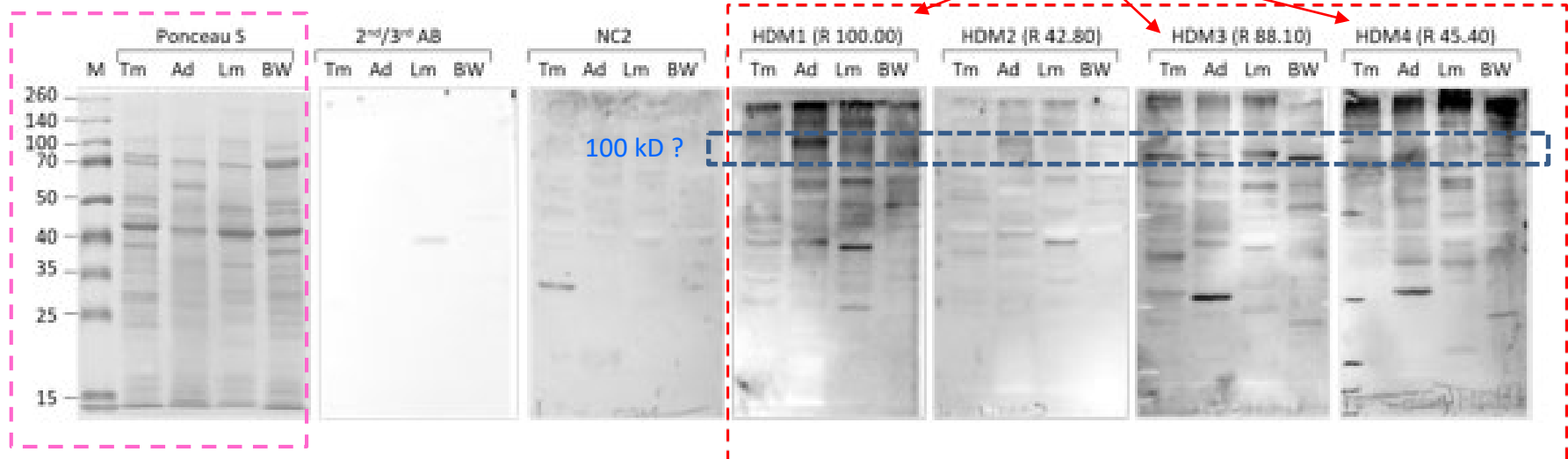
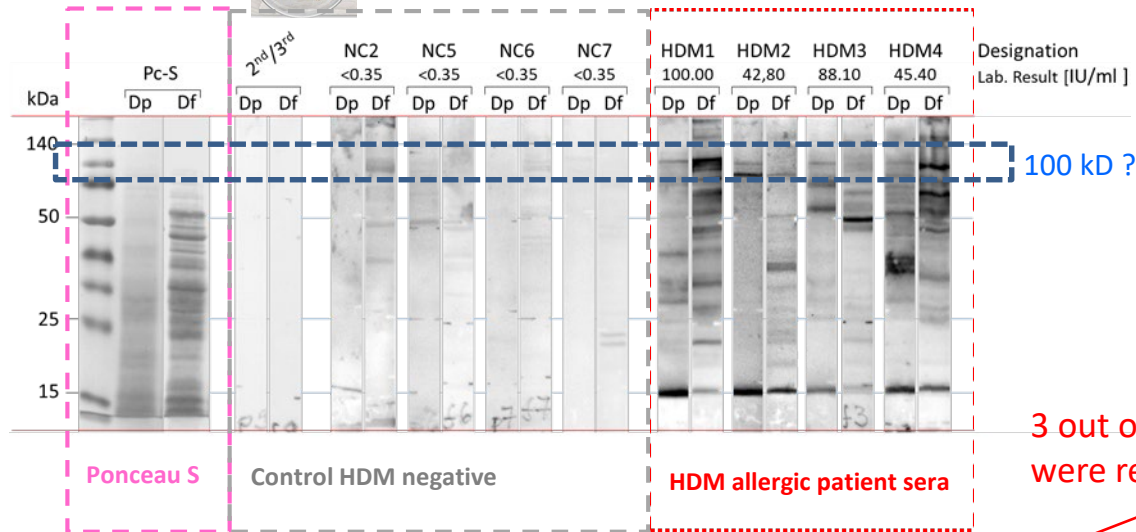


So far unknown 100 kD band
 is detected by IgE from the sera of
 HDM allergic patients as well

HDM IgE are crossreactive to IEF fractions of insect proteins



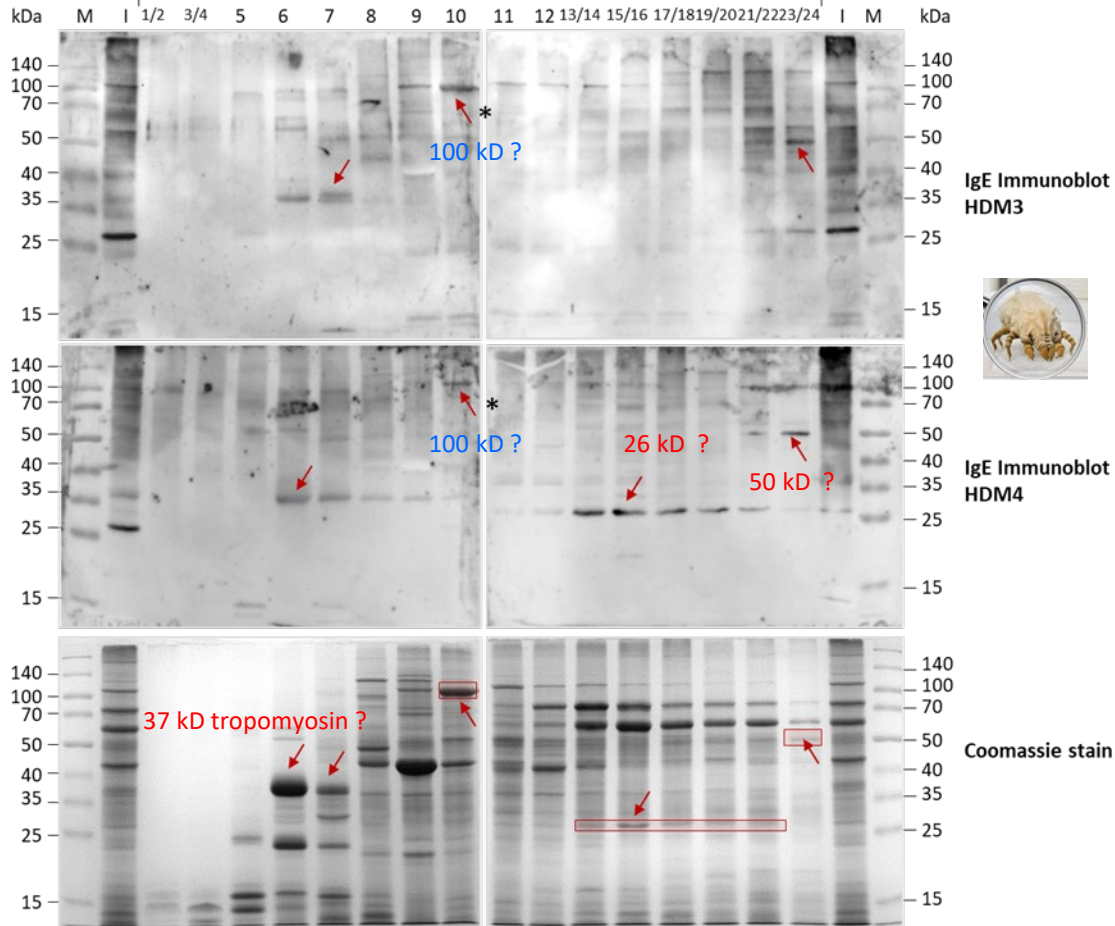
Dermatophagoides pteronyssinus (Dp) and *D. farinae* (Df); 20 µg / lane



House cricket proteins are bound by IgE from sera of HDM allergic patients



OFFGEL-Fraction



So far unknown 100 kD band is detected by IgE from the sera of crustacean allergic patients as well

Other potential allergens from House Cricket bound by IgE from sera of HDM allergic patients

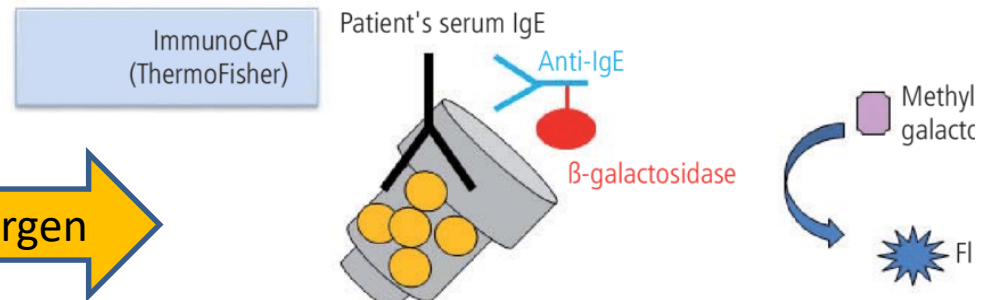
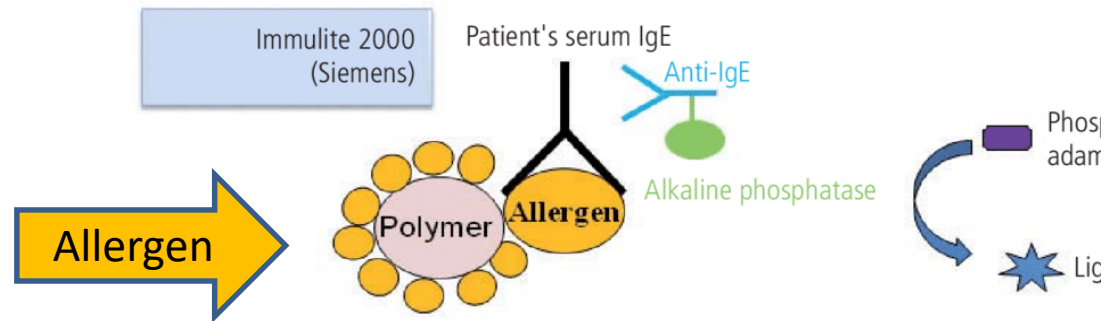
3. Summary

- Allergen Fractionation**
- IgE Profiling – DigiWest Allergen**

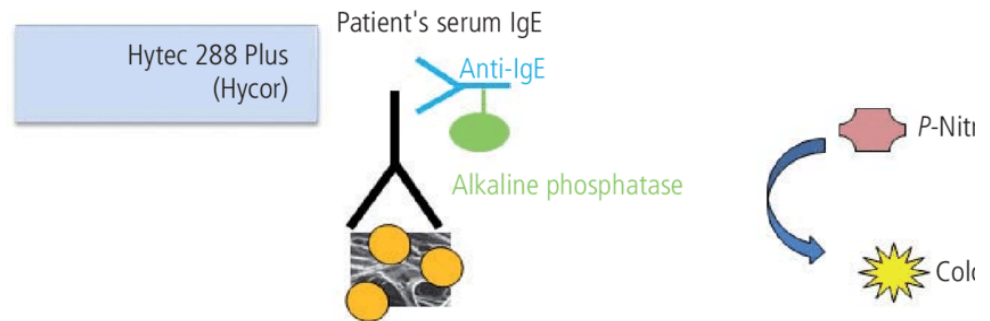
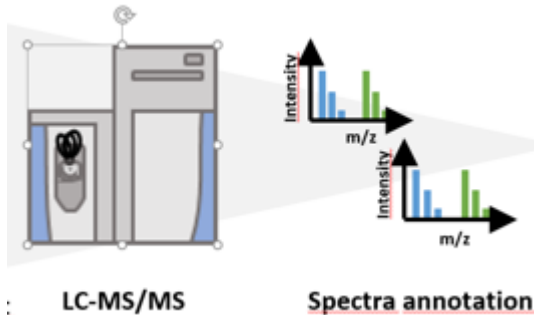
Summary 1: Fast access to fully characterized relevant natural allergens for diagnostic IgE profiling

Pure natural allergens
Application in established Allergy Diagnostics

Fractionation



Full Characterization



Multiplexed Allergy Screening ImmunoCAP™ ISAC™

ThermoFisher
SCIENTIFIC

- Array based IgE FIA
- 112 recombinant allergens , triplicates
- Total IgE

Pure natural allergens
Application in modern
multiplexed Allergy Diagnostics

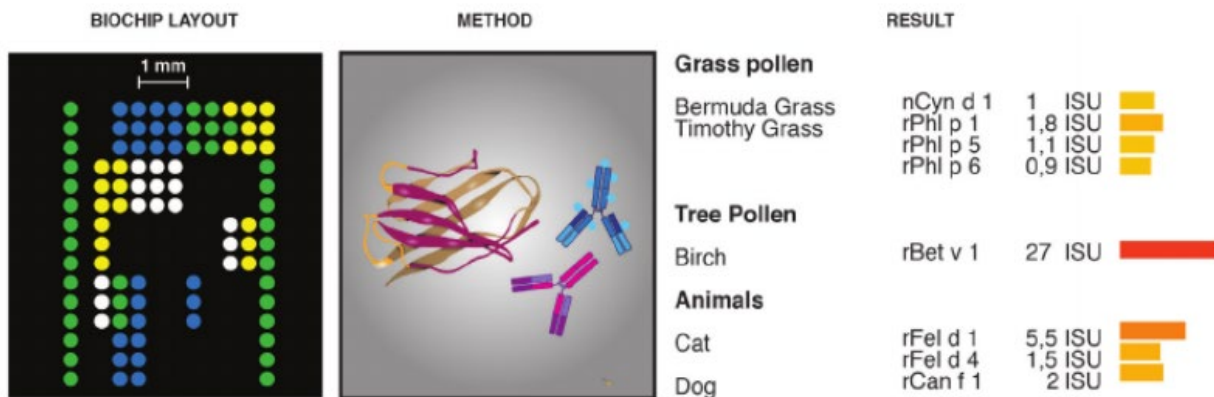
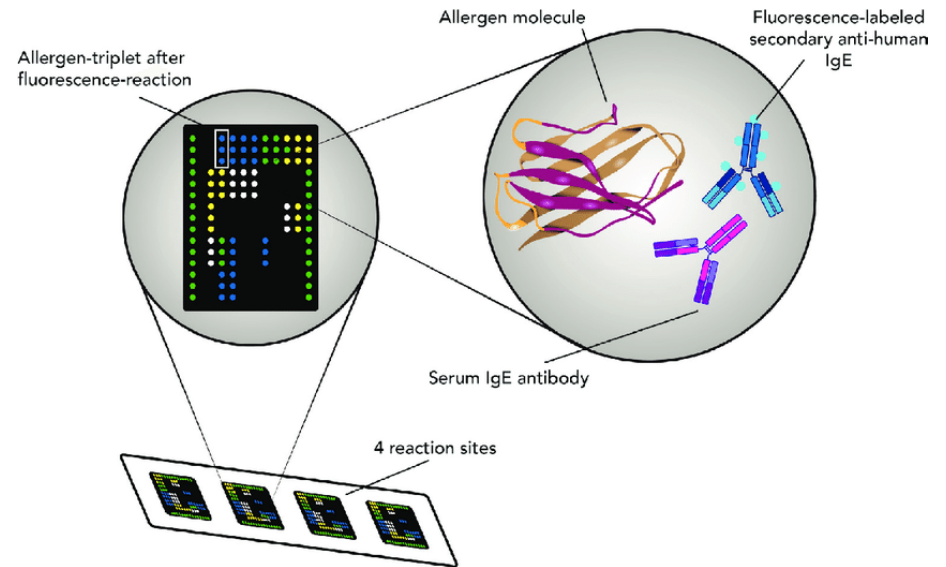


Figure 8. ISAC biochip layout. Results are reported in arbitrary units named ISAC-Standardized Units (ISU).

Source: Salazar, Alberto & Velazquez Soto, Henry & Ayala-Balboa, Julio & Jiménez-Martínez, María C. (2017). Allergen-Based Diagnostic: Novel and Old Methodologies with New Approaches. 10.5772/intechopen.69276.

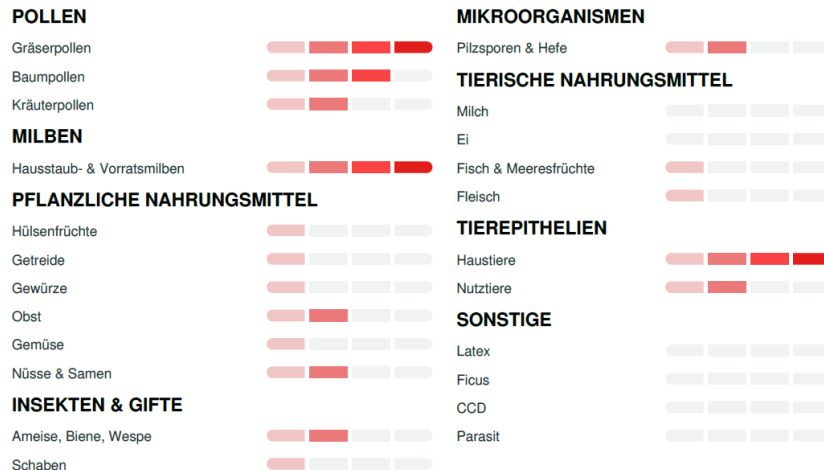
Multiplexed Allergy Screening Allergy Explorer - ALEX 2



- Nano Bead Array IgE ELISA
- 15 predefined allergen panels, 295 allergens
- 117 extracts & 178 molekular antigens
- Total IgE
- Blocks cross reactives carbohydrate determinants
- On-demand panels possible
- $\geq 200 \mu\text{l}$ of blood / $\geq 120 \mu\text{l}$ capillary blood with pre-dilution



Laborbericht: Zusammenfassung der nachweisbaren Sensibilisierungen






Pure natural allergens
Application in modern
multiplexed Allergy Diagnostics



Summary 2: Patient Risk Patterns for cross-reactive allergens

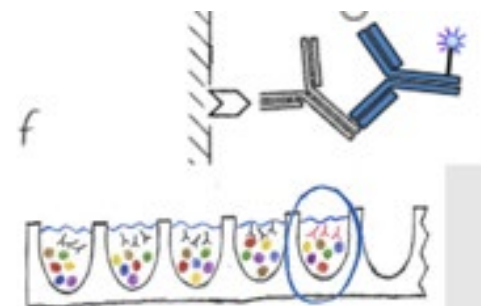
24 sera + 2 anti-tropomyosin abs

Internal Design.	Laboratory Parameter	Interpretation	Result [IU/ml]										
				Pm	Cp	Cc	Nn	Dp	Df	Tm	Ad	Lm	BW
NC1	slgE Peanut (f13)	negative	0.35	-	-	-	-			-	-	-	-
NC2	slgE Peanut (f13)	negative	0.35	-	+	-	-	-	+	+	-	-	-
NC3	slgE D. pteronyssinus (d1)	negative	0.35										
NC4	slgE D. pteronyssinus (d1)	negative	0.35										
NC5	slgE Shrimp (f24)	negative	0.35	-	-	-	-	-	-	-	-	-	-
NC6	slgE Shrimp (f24)	negative	0.35	-	-	-	-	-	-	-	+	+	+
NC7	slgE Shrimp (f24)	negative	0.35	+	-	-	-	-	+	-	-	-	-
NC8	slgE Shrimp (f24)	negative	0.35										
CrSr1	slgE Crab (f23)	positive	11.40	++	+	+	+			+++	+	+	+
CrSr2	slgE Crab (f23)	positive	29.00	+++	+++	+++	+++			+++	+++	+++	+++
CrSr3	slgE Crab (f23)	positive	13.40	++	++	+	+			-	+	+	+
CrSr4	slgE Crab (f23)	positive	100.00	++++	++++	++++	++++			++++	++++	++++	++++
Cr1	slgE Lobster (f80)	positive	2.51	++	++	+	+			-	-	-	-
Cr2	slgE Crab (f23)	positive	7.50	++	++	++	++			+	+	+	+
Cr3	slgE Crab (f23)	positive	7.50	++	++	++	+			++	++	++	++
Cr4	slgE Crab (f23)	positive	7.50	++	++	+	+			++	++	++	++
Cr5	slgE Crab (f23)	positive	12.40	+	+	++	-			++	±	±	±
Sr1	slgE Shrimp (f24)	positive	2.70	++	-	-	-			+	+	+	+
Sr2	slgE Shrimp (f24)	positive	2.97	+	+	+	-			+	+	+	+
Sr3	slgE Shrimp (f24)	positive	3.10	++	-	-	+			+	+	+	+
HDM1	slgE D. pteronyssinus (d1)	positive	100.00					+++	+++	++	++	++	++
HDM2	slgE D. pteronyssinus (d1)	positive	42.80					++	++	-	-	-	-
HDM3	slgE D. pteronyssinus (d1)	positive	88.10					++	++	+	+	+	+
HDM4	slgE D. pteronyssinus (d1)	positive	45.40					+++	+++	++	++	++	++

4 food insects



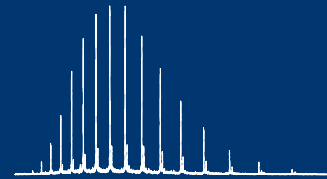
2 House dust mites



260 immunoblots
 260 x 24 fraction blots
DigiWest Allergen
 > 6500 immunoblot

Thank you for the good collaboration

..... in difficult Corona times ..!



HSAS

Eva-Maria Ladenburger
Robin Kretz
Larissa Walter
Henry Kit Carson
Sara Schweier

...

BfR

Hermann Broll
Christiano Garino

...

Charité

Kirsten Beyer
Lara Meixner
Stephanie Heller

....

HOT Screen GmbH

Manfred Schmolz
Marleen Willig

....

Signatope

Oliver Pötz
Hannes Planatscher
Tobias Meisinger
Andreas Steinhilber
Robin Kretz

.....

Nestlé

Marie-Claude Robert
Michael Affolter
Christoph Reh



NMI

Katja Schencke-Layland

Thomas O. Joos

Jens Göpfert

....

Nicole Schneiderhan-Marra

Markus Templin

Annette Döttinger

Felix Ruoff

Simon Fink

AllergenPro



Grant No: 281A304D18 **Nina Müller**

09/2020 – 02/2024