

APPENDICES

APPENDIX 1: Questionnaire used in the Skin Prick Test

FOOD ALLERGY / HYPERSENSITIVITY QUESTIONNAIRE

NAME: R/N No:
AGE: RACE:
SEX: TEL No.:

SYMPTOMS OF FOOD ALLERGY:

- I. RESPIRATORY: 1. Asthma
2. Rhinitis : a. Rhinorrhoea
b. Sneezing
c. Itchy nose
- II. GIT: Nausea
Vomiting
Diarrhoea
- III. OTHERS: Headache
Itchy Eyes
Urticaria
Eczema

FAMILY HISTORY OF ALLERGY

Father Mother Siblings Children

ACCOMODATION HISTORY

1. Carpets at home
2. Pets
3. Household pests(rats/cockroaches)
4. Air-conditioning(at home/work)
5. On staying away from home, symptoms become (better/worse/absent)

SYMPTOMS ARE PROVOKED AND/OR AGGRAVATED BY

1. House dust
2. Temperature changes
3. Animal dander
4. Food
 - i. Dairy products
 - ii. Seafood.....
 - iii. Meat
 - iv. Others

APPENDIX 2: Nucleotide profile of Der p 10 DNA as sequenced by the ABI system

gtGgcGaCCTCTCCaAAATCGGATCTGGTTCGCGGTGGATCCaTGGAGGCCATCAAAAAGAAAAATGCAGGCAATGAAGC
TCGAAAAAGATAATGCTATCGATCGAGCTGAAATCGCCGAACAAAAGCCGTGATGCTAATCTTCGTGCTGAAAAAGTCC
GAAGAAGAAGTACGTGCATTACAGAAAAAATCCAAAAATCGAGAAATGAATTGGATCAGGTTCAAGAACAAATTATCGGC
TGCCAAATACAAAATTGGAAGAAAAGGAAAAGCCCTGCAAACTGCTGAAGGTGATGTTGCAGCATTTGAATCGTCTGATTC
AATTGATCGAAGAAGATTGGAACGATCAGAAGAACGACTTAAATTTGCAACAGCTAAATTGGAAGAAGCATCACAAATCT
GCCGATGAATCCGAACGTATCGCAAAAATGTTTGGAAACATCGTTCCATTACCGATGAAGAACGTATGGAAGGTTTGGAAAA
TCAATTAAGAAGAACACGTATGATGGCTGAAGATGCTGATAGAAAATATGATGAAGTTGCACGTAAATTAGCAATGGTTG
AAGCCGATTTAGAACGTGCTGAAGAACGTGCCGAACCTGGTGAATCGAAAAATTTGCGAACCTTGAGGAAGAAATTGCGTGT
TTGGTGAATAATCTAAAAATCATTTGGAAGTCAGTGAAGAAAAAGCACAAACGTGAAGAAAGCACATGAACACAGATCCG
TaTTATGACACCCAACTTAAGAAAGCTGAAGCACGTGCCGAAATTCgCTTGAACGATCAAGTgCAAAA

GGTaTACCGTCaTCAACCGAAACGGCGAGGCAGATCGTCAGTcAGTCACGATGCGGCCGCTCGAGTCGACCCTCTAGATT
AATAACCAAGTAAGTTCGGCAAAATGTTTGGTCCAAATTCATCGGAAATGGATTTATATTTTCTTTTTCATGGACCAATTCA
TCTTCCAAACGATCAACTCTTTTGTAAATTTTGTCACTGATCGTTACGCAATTCGGCACAGTCTCAGCTTCTTTAAAGT
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CGGATTCAACGGCAGATTGGATGCTTCTTCCAAATTTAACTGTGCAATTTAAAGTCGTTCTTCTGATCGTTCCAAAATCTTc
TTCGATCAATTGAATACGACgATTCAATGCTTGGAACATCaCCTTCAGCgGTTTGCAGGGCTTTTTCCTTTCTTTCCAATTTC
GATGG

Appendix 3: Result generated by the BLAST in the analysis of the nucleotide profile of Der p 10 DNA as sequenced by the ABI system

```
-----
> Program Blastn
> DataLib GenBank
> Descriptions 100
> Alignments 100
  > View 0
  > Filter T
> Gap_Open 5
> Gap_Extend 2
  > GI F
> Gap_Align T

> What is the % of similarity?
-----
***MSDEBUG Information***
/opt/bin/blastall -p blastn -d /xp01/blast2/db/blastdb/genbank_A -i 20030601013529794.query -W
11 -G 5 -E 2 -m 0 -I F -g T -v 100 -b 100 -F T -K 100 >> 20030601013529794.out 2>
20030601013529794.err
*****
BLASTN 2.1.2 [Oct-19-2000]

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs", Nucleic Acids Res. 25:3389-3402.
Query= What is the % of similarity?
      (1518 letters)
      Database: genbank_A
      24,027,936 sequences; 31,099,264,455 total letters

Searching.....done

Score  E  Sequences producing significant alignments:          (bits) Value
(AF016278) Dermatophagoides pteronyssinus tropomyosin mRNA, com... 1386 0.0
(Y14906) Dermatophagoides pteronyssinus mRNA for tropomyosin.      1314 0.0
Complete cds.
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Score = 1386 bits (699), Expect = 0.0
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Strand = Plus / Plus

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Query: 164 gaagaagtagctgcattacagnnnnnnntccaacaatcgagaatgaattggatcaggtt 223
|||||
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Query: 164 gaagaagtagctgcattacagnnnnnnntccaacaatcgagaatgaattggatcaggtt 223

|||||

Sbjct: 294 gaagaagtagctgcattacagaaaaaaaccaacaatcgagaatgaattggatcaggtt 353

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Sbjct: 594 atggaaggtttgaaaaatcaattaaagaagcacgtatgatggctgaagatgctgata 653

Query: 524 aaatatgatgaagttgcacgtaaattagcaatggttgaagccgatttagaacgtgctgaa 583

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Sbjct: 654 aaatatgatgaagttgcacgtaaattagcaatggttgaagccgatttagaacgtgctgaa 713

Query: 584 gaacgtgccgaaactggtgaatcgaaaattgtcgaactgaggaagaattgcgtgtgtt 643

|||||

Sbjct: 714 gaacgtgccgaaactggtgaatcgaaaattgtcgaactgaggaagaattgcgtgtgtt 773

Query: 644 ggtaataatctaaatcattggaagtcagtgaagaaaaagcacaacaacgtgaagaagca 703

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Sbjct: 774 ggtaataatctaaatcattggaagtcagtgaagaaaaagcacaacaacgtgaagaagca 833

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|||||

Sbjct: 834 catgaacaacagatccgtattatgacaacaaactaaagaagctgaagcacgtgccgaa 893

Query: 763 ttcgcttgaacgatcagtgcaaaa 786

|||||

Sbjct: 894 ttcgc-tgaacgatcagtgcaaaa 916

Score = 1253 bits (632), Expect = 0.0

Identities = 644/648 (99%)

Strand = Plus / Minus

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|||||

Sbjct: 1028 ttaataaccagtaagttcgcaaatgtttgtccaattcatcgaaatggattatattt 969

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Query: 1045 gatctgtgtcatgtgcttctcacgtgttgctttttcttactgacttccaatga 1104
 |||||
 Sbjct: 848 gatctgtgtcatgtgcttctcacgtgttgctttttcttactgacttccaatga 789

Query: 1105 tttagattattaccaacaacacgcaattctcctcaagtcgacaattttcgattcacc 1164
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 Sbjct: 608 ttccaaaccttcacatggttctcatcggtaatggaacgatgtccaacattttcgcat 549

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 Sbjct: 488 tcgttctctgatcgttccaatcttcttcgatcaattgaatacagcattcaatgctgg 429

Query: 1465 aacatcaccttcagcagtttgagggttttcttctttccaatt 1512
 |||||
 Sbjct: 428 aacatcaccttcagcagtttgagggttttcttctttccaatt 381

>(Y14906) Dermatophagoides pteronyssinus mRNA for tropomyosin.
 Length = 855
 Score = 1314 bits (663), Expect = 0.0
 Identities = 724/744 (97%), Gaps = 2/744 (0%)
 Strand = Plus / Plus

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 |||||
 Sbjct: 1 atggaggccatcaagaataaaatgcaggcaatgaagctcgaaaaagataatgctatcgat 60

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 Sbjct: 61 cgagctgaaatgccgaacaaaaagcccgtgatgctaattctctgctgtaaaagtccgaa 120

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Sbjct: 601 ggtaataatctaaaatcattggaagttagtgaagaaaaagcacaacaacgtgaagaagca 660

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|||||

Sbjct: 721 ttcg-tgaacgatcagtgc meta 743

Score = 1197 bits (604), Expect = 0.0

Identities = 637/648 (98%)

Strand = Plus / Minus

APPENDIX 4 : Peptide synthesis schedule as generated by PepMaker programme

- (i) Page 1 - Description of protein sequence to be synthesized
- (ii) Page 2 - Peptides to be synthesized on each pin
- (iii)Page 3 - Coupling schedule for 1st residue on each pin : aa to be weighed and the solution needed

N.B.: Pages 4 to18 - (not shown); contain the rest of the coupling and dispensing schedule for 2nd till 8th residues.

Description:

Mite tropomyosin with 299 residues synthesized as octamer peptides overlapping by four.

8-mer peptides based on the sequence DFIO.ASC
Peptide spacing increment is 4

Segment 1: 73 peptides starting at residue I

First peptide: [FFFVAAKQ]

Last peptide: [LDQTFTEL]

Protein sequence: DFIO.ASC (299 residues)

1:	FFFVAAKQQQ	QPSTKMEAIK	KKMQAMKLEK	DNAIDRAEIA	EQKARDANLE
51:	AEKSEEEVRA	LQKKIQQIEN	ELDQVQEQLS	AANTKLEEKE	KALQTAEGD\
101:	AALNRRILQI	EEDLERSEER	LKIATAKLEE	ASOSADESES	MRKMLEHRSI
151:	TDEERMDGLE	NQLKEARMMA	EDADRKYDEV	ARKLAMVEAI	LERAEERAEI
201:	GESKIVELEE	ELRVVGNNLK	SLEVSEEKAQ	QREEAYEQQI	RIMTAKLKGA
251:	EARAEEFAERS	VQKLQKEVDR	LEDELVHERE	KYKSISDELI:	---

Amino Acid set to be used - AASET3

aaset I: Free acid L-Fmoc amino acids - DIC/HOBt chemistry

Positive Control Peptide PLAQ

Negative Control Peptide GLAQ

Number of copies of each peptide 2

Schedule based on a 150 microlitre fill/well
(Well concentration is 60 mM)

Details of pins used in the synthesis

Pin Batch No.	
Acid level	 microMole
HMD level	 mMole
Beta-Alanine	 mMole

Blocks A to B = 2 Blocks

Input Data checked and found to be correct
Synthesis authorized to proceed

GENERAL NET SYNTHESIS SCHEDULE

Chemistry Group I data for synthesis coupling

Amino terminus is printed on the left

1 A 1(1,2)PLAQ <	49 B 1(1,2)PLAQ <
2 A 2(1,2)GLAQ <	50 B 2(1,2)GLAQ <
3 A 3(1,2)FFFVAAKQ<	51 B 3(1,2)AMVEADLE<
4 A 4(1,2)AAKQQQQP<	52 B 4(1,2)ADLERAE<
5 A 5(1,2)QQQPSTKM<	53 B 5(1,2)RAEERAET<
6 A 6(1,2)STKMEAIK<	54 B 6(1,2)RAETGESK<
7 A 7(1,2)EAIKKMQ<	55 B 7(1,2)GESKIVEL<
8 A 8(1,2)KKMQAMKL<	56 B 8(1,2)IVELEEEL<
9 A 9(1,2)AMKLEKDN<	57 B 9(1,2)EEELRWG<
10 A10(1,2)EKDNAIDR<	58 B10(1,2)RWGNNLK<
11 A11(1,2)AIDRAEIA<	59 B11(1,2)NNLKSLEV<
12 A12(1,2)AEIAEQKA<	60 B12(1,2)SLEVSEEK<
13 A 1(3,4)EQKARDAN<	61 B 1(3,4)SEEKAQQR<
14 A 2(3,4)RDANLRAE<	62 B 2(3,4)AQQREEAY<
15 A 3(3,4)LRAEKSEE<	63 B 3(3,4)EEAYEQQI<
16 A 4(3,4)KSEEEVRA<	64 B 4(3,4)EQQIRIMT<
17 A 5(3,4)EVRALQKK<	65 B 5(3,4)RIMTAKLK<
18 A 6(3,4)LQKKIQQI<	66 B 6(3,4)AKLKEAEA<
19 A 7(3,4)IQQIENEL<	67 B 7(3,4)EAEARAEF<
20 A 8(3,4)ENELDQVQ<	68 B 8(3,4)RAEFAERS<
21 A 9(3,4)DQVQEQLS<	69 B 9(3,4)AERSVQKL<
22 A10(3,4)EQLSAANT<	70 B10(3,4)VQKLQKEV<
23 A11(3,4)AANTKLEE<	71 B11(3,4)QKEVDRL<
24 A12(3,4)KLEEKEKA<	72 B12(3,4)DRLEDELV<
25 A 1(5,6)KEKALQTA<	73 B 1(5,6)DELVHERE<
26 A 2(5,6)LQTAEGDV<	74 B 2(5,6)HEREKYKS<
27 A 3(5,6)EGDVAAALN<	75 B 3(5,6)KYKSISDE<
28 A 4(5,6)AALNRRIQ<	76 B 4(5,6)ISDELDQT<
29 A 5(5,6)RRIQLIEE<	
30 A 6(5,6)LIEEDLER<	
31 A 7(5,6)DLERSEER<	
32 A 8(5,6)SEERLKIA<	
33 A 9(5,6)LKIATAKL<	
34 A10(5,6)TAKLEBAS<	
35 A11(5,6)EEASOSAD<	
36 A12(5,6)OSADESER<	
37 A 1(7,8)ESERMCRM<	
38 A 2(7,8)MRKMLEHR<	
39 A 3(7,8)LEHRSITD<	
40 A 4(7,8)SITDEERM<	
41 A 5(7,8)EERMDGLE<	
42 A 6(7,8)DGLNQKL<	
43 A 7(7,8)NQLKEARM<	
44 A 8(7,8)EARMMAED<	
45 A 9(7,8)MAEDADRK<	
46 A10(7,8)ADRKYDEV<	
47 A11(7,8)YDEVARKL<	
48 A12(7,8)ARKLAMVE<	

Bulk solutions for activator and/or additives (154 wells)

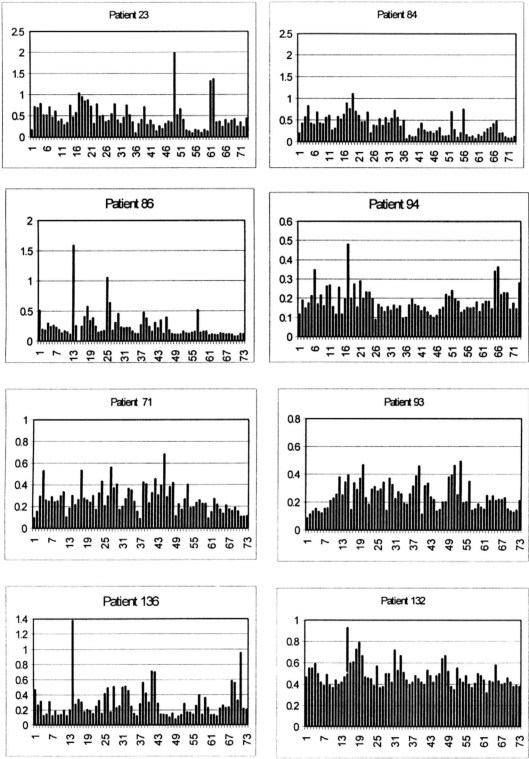
Activator : DIC requires 208.2 mg in 5.5 ml of DMF

Additive : HOBt requires 296.5 mg in 21.5 ml of DMF

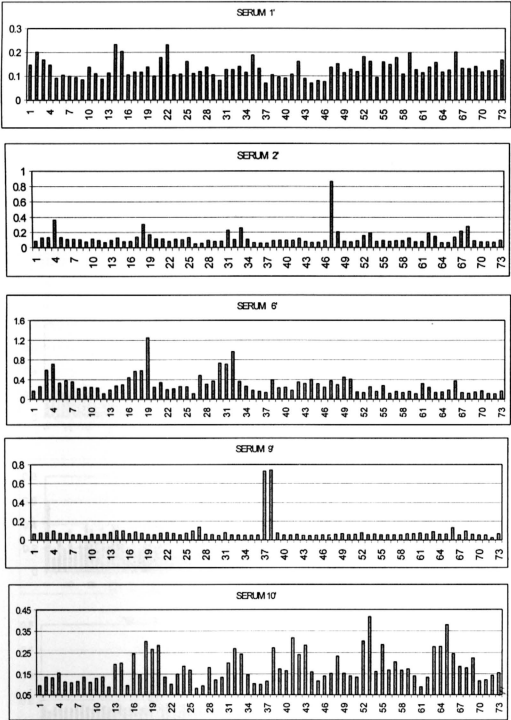
WEIGHTS FOR INDIVIDUAL AMINO ACID SOLUTIONS

AA #	Amino acid description	Batch	Weight (mg)		DMF (ml)	DIC (ml)	HOBt (ml)
A 14	-L-Ala-OH.H2O		47.3		0.00	0.48	1.91
D 6	-L-Asp(OtBu) -OH		28.8		0.00	0.23	0.93
E 22	-L-Glu(OtBu) -OH.H2O	O	96.2		0.00	0.72	2.89
F 2	-L-Phe-OH		12.9		0.00	0.11	0.44
G 2	-Gly-OH		9.9		0.00	0.11	0.44
I 2	-L-Ile-OH		18.3		0.00	0.17	0.69
K 16	-L-Lys(Boc) -OH		75.8		0.00	0.54	2.16
L 16	-L-Leu-OH		57.2		0.00	0.54	2.16
M 8	-L-Met-OH		32.9		0.00	0.29	1.18
N 6	-L-Asn(Trt) -OH		41.8		0.00	0.23	0.93
P 2	-L-Pro-OH		11.3		0.00	0.11	0.44
Q 8	-L-Gln(trt) -OH		54.0		0.00	0.29	1.18
R 12	-L-Arg(PMC) -OH.31PE	E	87.0		0.00	0.42	1.67
S 8	-L-Ser(tBu) -OH		33.9		0.00	0.29	1.18
T 8	-L-Thr(tBu) -OH		35.2		0.00	0.29	1.18
V 10	-L-Val-OH		36.2		0.00	0.36	1.42
Y 2	-L-Tyr(tBu) -OH		15.3		0.00	0.11	0.44
					0.00	5.32	21.27

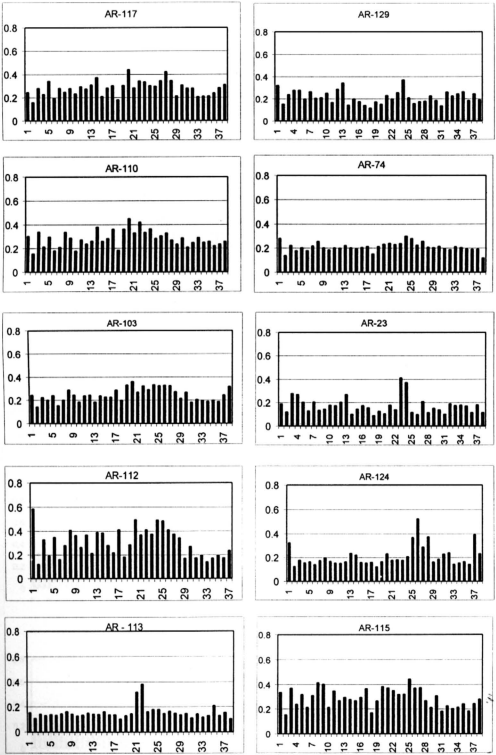
APPENDIX 5a: IgE binding profiles of 8 allergic rhinitis patients, to 73 linear peptides representing the mite tropomyosin, Der f 10. Absorbance was obtained at 405/492 nm.



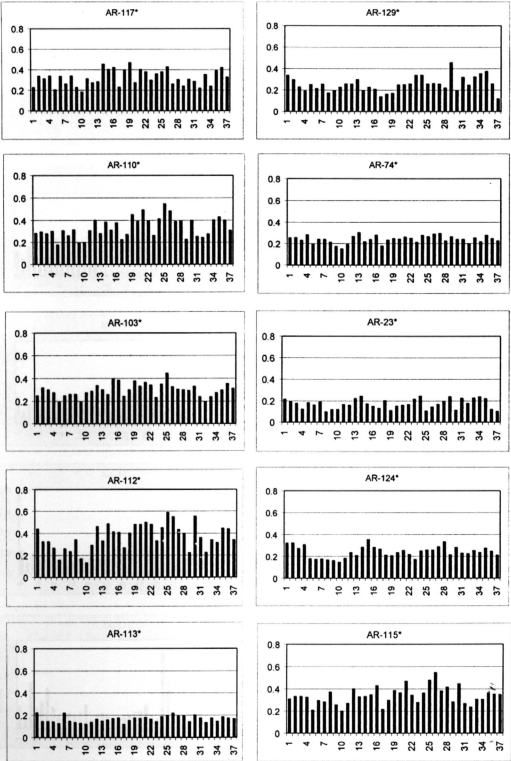
APPENDIX 5b: IgE binding profiles of 5 asthmatic patients, to 73 linear peptides representing the mite tropomyosin, Der f 10. Absorbance was obtained at 405/492 nm.



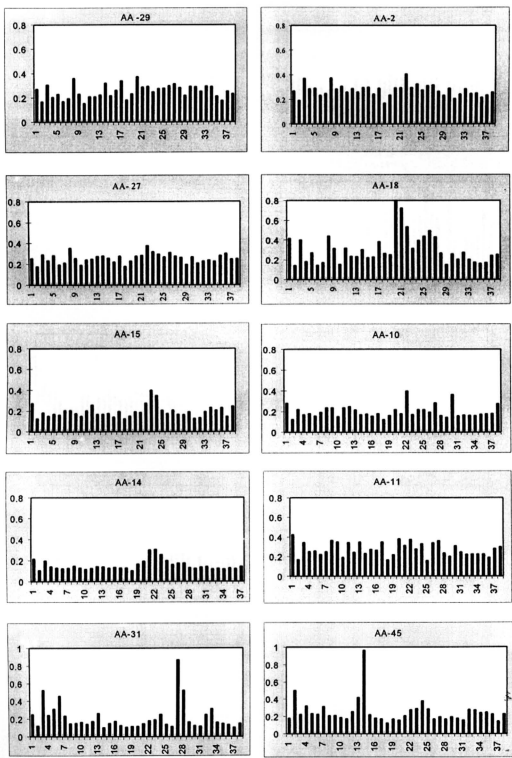
APPENDIX 6a: IgE-binding reactivity profiles of 10 allergic rhinitis patients to the first frame chimeric peptides of Der f 10. 38 pin-bound peptides (x-axis) of 30-mers were tested by using pinELISA and the OD (y-axis) was read at 450 nm wavelength.



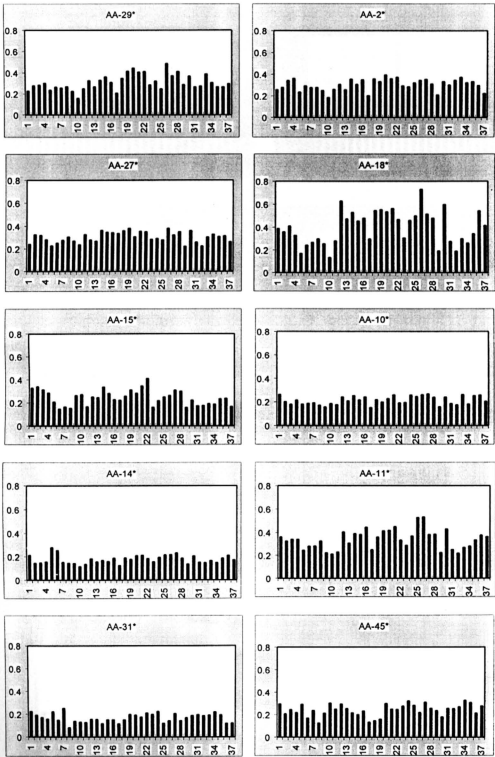
APPENDIX 6b: IgE-binding reactivity profiles of 10 allergic rhinitis patients to the second frame chimeric peptides of Der f 10. 37 pin-bound peptides (x-axis) of 30-mers were tested by using pinELISA and the OD (y-axis) was read at 450 nm wavelength.



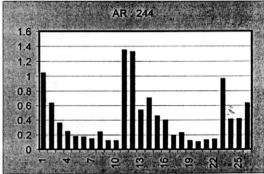
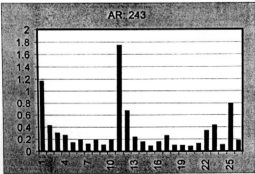
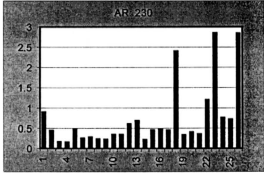
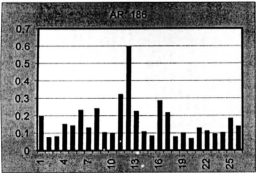
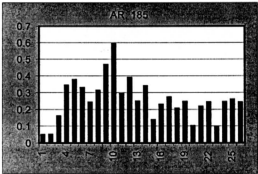
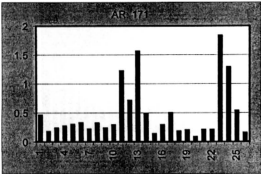
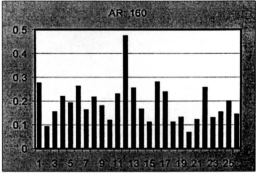
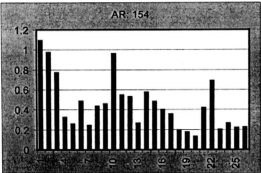
APPENDIX 7a: IgE-binding reactivity profiles of 10 asthmatic patients to the first frame chimeric peptides of Der f 10. 38 pin-bound peptides (x-axis) of 30-mers were tested by using pinELISA and the OD (y-axis) was read at 450 nm wavelength.



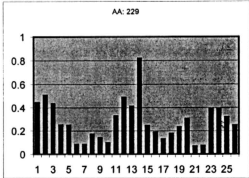
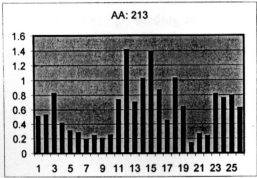
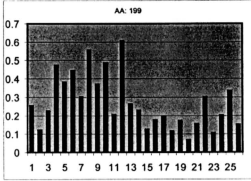
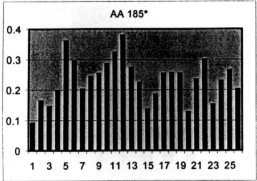
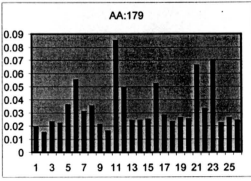
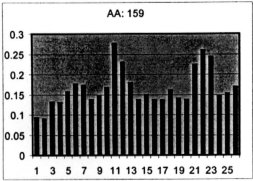
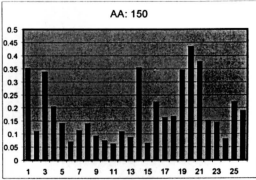
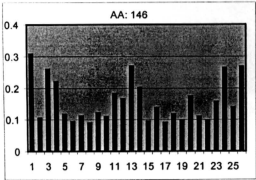
APPENDIX 7b: IgE-binding reactivity profiles of 10 asthmatic patients to the second frame chimeric peptides of Der f 10. 37 pin-bound peptides (x-axis) of 30-mers were tested by using pinELISA and the OD (y-axis) was read at 450 nm wavelength.

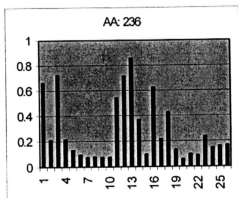
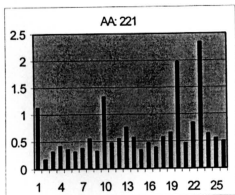
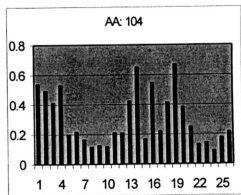
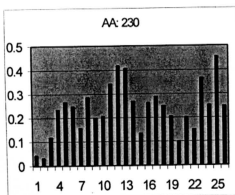
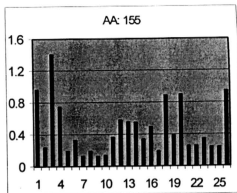
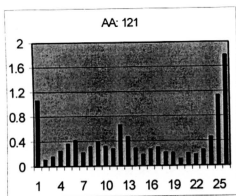


APPENDIX 8a: The collective values of IgE binding reactivities of 8 allergic rhinitis patients against the 134 amino acid residues of Blo t 5 allergen, synthesised as 12-mer peptides. A cut-off at OD 0.4 differentiates high-binding peptides from the non-binding ones.



APPENDIX 8b: The IgE-binding reactivities of 8 out of 14 asthmatic patients, with positive SPT to *Blomia tropicalis* allergen. 26 pins with bound 12 mer peptides were tested against these sera in the pinELISA. Another 6 reactivity profiles are shown on the following page.





N.B. : The IgE-binding reactivities of another 6 out of 14 asthmatic patients, with positive SPT to *Blomia tropicalis* allergen. 26 pins with bound 12-mer peptides were tested against these sera in the pinELISA. Another 8 reactivity profiles are shown on the previous page.