VIII. APPENDIXES

APPENDIX A

Recipes

A. Staining dyes

1. Wright-Giemsa stain

Reagents

1. Wright's stain powder	4.50	gm
2. Giemsa powder	0.45	gm
3. Absolute methanol	2.5	L

Directions

Dissolve Wright' stain powder and Giemsa powder with absolute methanol, mix thoroughly. Incubate at 37 °C for 5 day or more, filtered before use.

2. Toluidine blue stain, pH 4.1

Reagents

1. Toluidine blue O	0.5	gm
2. Sodium phosphate dibasic. 12 H_2O	28.6	gm
3. Citric acid	12.6	gm
4. Distilled water	1000	ml

Directions

Dissolve the reagents with distilled water, mix thoroughly. Adjust pH to 4.1 by using 1 N HCl or 1 N NaOH, store at room temperature.

3. Alcian blue/Safranin stain

Reagents

1. Alcian blue 8 GX	0.36	gm
2. Safranin O	0.18	gm

3. Ferric ammonium sulphate	0.48	gm
4. Acetate buffer (pH 1.42)	100	ml

Directions

Dissolve the reagents with acetate buffer, mix thoroughly. The solution was stored at room temperature.

4. Acetate buffer, pH 1.42

Reagents

1. 1 N HCl	60	ml
2. 1 N Sodium acetate	50	ml
3. Distilled water	250	ml

Directions

Dissolve all substances with distilled water; adjust pH to 1.42 by using 1 N HCl or 1 N NaOH. The solution was stored at room temperature.

5. 1 N Sodium acetate

Reagents

1. Sodium acetate	82.04	gm
2. Distilled water	1000	ml

Directions

Suspend sodium acetate in distilled water; mix thoroughly. The solution was stored at room temperature.

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6. 95 % ethyl alcohol

Reagents

1. Absolute ethanol	95	ml
2. Distilled water	5	ml

Directions

Dissolve absolute ethanol with distilled water. The solution was stored at room temperature.

B. Culture media

1. Blood agar (BA)

Reagents

1. Trypticase soy agar (TSA) 40 gm
2. Distilled water 1000 ml

Directions

Dissolve TSA with distilled water, mix completely by boiling. Sterilize by autoclaving at 121 °C for 15 min. Leave to 50-55 °C; add 5 % sterile human expired blood, then pour plates with continuous shaking.

2. Mannitol salt agar

Reagents

1. Mannitol salt agar 108.0 gm
2. Distilled water 1000 ml

Directions

Suspend Mannitol salt agar in distilled water and heat in the boiling water bath or in the stream-pot until completely dissolved. Sterilize by autoclaving for 15 min at 121 °C. Then pour plates with continuous shaking.

3. MacConkey agar

Reagents

1. MacConkey agar 51.5 gm
2. Distilled water 1000 ml

Directions

Suspend MacConkey agar in distilled water and heat until completely dissolve. Sterilize by autoclaving for 15 min at 121 °C.

4. Mueller hinton agar

Reagents

1. Mueller hinton agar 38.0 gm

2. Distilled water	1000	ml

Directions

Suspend 38 gm of powder in 1 liter of distilled water. Bring to boil until the medium dissolved completely. Sterilize by autoclaving at 121 °C for 15 min.

5. Storage media

Reagents

1. Oxiod tryptone soya broth	3.0	gm
2. Glucose	0.5	gm
3. Skim milk powder	2.0	gm
4. Glycerol	10	ml

Directions

Distilled water was added to 100 ml. The solution was dispended in 10 ml amounts into each tube and autoclaved at 15 lb/in for 10 min, stored at 4-6 °C.

C. Enzyme-linked immunosorbent assay (ELISA) reagents

1. Phosphate buffer saline (PBS), pH 7.4

Reagents

1. NaCl	8.00	gm
2. Na_2HPO_4	1.44	gm
3. KCl	0.20	gm
4. KH_2PO_4	0.24	gm
5. DW	1000	ml

Directions

Dissolve and mix thoroughly. Adjust the pH to 7.4 with 1 N HCl or 1 N NaOH. The solution was stored at room temperature.

2. Washing solution (PBST)

Reagents

1. PBS	1.0	L
2. Tween-20	0.5	ml

Directions

Suspend PBS with 0.05 % Tween-20. Mix thoroughly. The solution was stored at room temperature.

3. Coating buffer (0.1 M $NaHCO_3$), pH 8.3

Reagents

1. $NaHCO_3$	4.2	gm
2. DW	500	ml

Directions

Dissolve and mix thoroughly. Adjust the pH to 8.3 with 1 N HCl or 1 N NaOH. Store at 4 °C.

4. Blocking solution (1 %BSA-PBST)

Reagents

1. Bovine serum albumin (BSA)	0.4	gm
2. PBST	40	ml

Directions

Dissolve BSA with PBST, mix thoroughly. Freshly prepare before use.

5. Citric acid, pH 4.0

Reagents

1. Citric acid	10.5	gm
2. Distilled water	500	ml

Directions

Suspend citric acid in distilled water, mix through completely dissolve. The solution was stored at 4 °C.

6. ABTS substrate

Reagents

1. ABTS	0.006	gm
2. Citric acid (pH 4.0)	6.25	ml
3. Distilled water	6.25	ml

4. 3 % H_2O_2	50	µl

Directions

Dissolve ABTS powder, citric acid with distilled water, keep in the dark. Add 3 % H_2O_2 before dispense to the well.

7. 1 N HCl

Reagents

1. Concentrate HCl	12.5	ml
2. Distilled water	150.0	ml

Directions

Suspend concentrate HCl in distilled water, mix thoroughly. The solution was stored at room temperature.

8. 1 N NaOH

Reagents

1. NaOH	4	gm
2. Distilled water	100	ml

Directions

Dissolve and mix thoroughly. The solution was stored at room temperature.

APPENDIX B

Sample size calculation

Sample size calculation

The sample size of each severity group was calculated by using more than two groups one way ANOVA (Norman and Streiner, 1994).

1. δ = max – min; $2.68 \times 10^4 - 4.25 \times 10^2 = 2.64 \times 10^4$
2. $d = \delta/s$, $s = \sqrt{\{ (n_1-1)S_1^2 + (n_2-1)S_2^2\}/ n_1+ n_2 -2}$

   $= 2.31 \times 10^4$

   $d = 1.14$
3. Effect size = $d \times 1/2\sqrt{k + 1/3(k-1)}$ = 0.47 ~ 0.05
4. Open table for sample size; 95%, $\alpha = 0.05$

   Sample size = 14

APPENDIX C

Results

Table 10. The severity, skin test results, optical density and concentration of anti house dust mite antibody specific to Der p 1 in AR patients.

No.	Severity score	OD	ng/ml
P1	14	0.549	375.528
P2	10	0.142	1.928
P3	4	0.118	0.000
P4	8	0.156	14.779
P5	4	0.126	0.000
P6	13	0.143	2.846
P7	9	0.113	0.000
P8	5	0.101	0.000
P9	13	0.145	4.681
P10	4	0.110	0.000
P11	3	0.097	0.000
P12	13	0.176	33.138
P13	8	0.125	0.000
P14	11	0.110	0.000
P15	4	0.133	0.000
P16	8	0.114	0.000
P17	13	0.371	212.135
P18	7	0.108	0.000
P19	4	0.100	0.000
P20	8	0.111	0.000
P21	4	0.101	0.000
P22	4	0.102	0.000
P23	13	0.990	780.338
P24	6	0.108	0.000
P25	3	0.118	0.000
P26	4	0.111	0.000
P27	4	0.112	0.000
P28	13	0.458	291.996
P29	4	0.117	0.000
P30	3	0.117	0.000

Table 10. The severity, skin test results, optical density and concentration of anti house dust mite antibody specific to Der p 1 in AR patients (continued).

No.	Severity score	OD	ng/ml
P30	3	0.117	0.000
P31	14	0.147	6.517
P32	3	0.268	117.588
P33	3	0.100	0.000
P34	7	0.098	0.000
P35	6	0.090	0.000
P36	4	0.106	0.000
P37	13	0.117	0.000
P38	13	0.175	32.220
P39	11	0.236	88.214
P40	10	0.163	21.204
P41	13	0.166	23.598
P42	13	0.435	270.883
P43	13	0.245	96.475
P44	4	0.337	180.925
P45	3	0.109	0.000
P46	13	0.105	0.000
P47	13	0.179	35.891
P48	13	2.208	1898.384
P49	4	1.039	825.317
P50	8	0.111	0.000
P51	10	0.129	0.000
P52	10	0.151	10.189
P53	13	0.142	1.928
P54	13	0.361	202.956
P55	7	0.141	1.010
mean	8.3	0.233	100.558
SD	4.0	0.330	297.898

Table 11. The severity, skin test results, optical density and concentration of anti house dust mite antibody specific to Der f 1 in AR patients.

No.	Severity score	OD	ng/ml
P1	14	0.715	527.905
P2	10	0.113	0.000
P3	4	0.114	0.000
P4	8	0.091	0.000
P5	4	0.093	0.000
P6	13	0.401	239.673
P7	9	0.099	0.000
P8	5	0.088	0.000
P9	13	0.177	34.055
P10	4	0.101	0.000
P11	3	0.093	0.000
P12	13	0.184	40.481
P13	8	0.111	0.000
P14	11	0.099	0.000
P15	4	0.094	0.000
P16	8	0.102	0.000
P17	13	0.453	287.406
P18	7	0.084	0.000
P19	4	0.087	0.000
P20	8	0.090	0.000
P21	4	0.087	0.000
P22	4	0.088	0.000
P23	13	0.096	0.000
P24	6	0.098	0.000
P25	3	0.114	0.000
P26	4	0.096	0.000
P27	4	0.095	0.000
P28	13	0.528	356.251
P29	4	0.091	0.000
P30	3	0.088	0.000

Table 11. The severity, skin test results, optical density and concentration of anti house dust mite antibody specific to Der f 1 in AR patients (continued).

No.	Severity score	OD	ng/ml
P31	14	0.124	0.000
P32	3	0.226	79.034
P33	3	0.116	0.000
P34	7	0.117	0.000
P35	6	0.082	0.000
P36	4	0.093	0.000
P37	13	0.133	0.000
P38	13	0.119	0.000
P39	11	0.089	0.000
P40	10	0.093	0.000
P41	13	0.097	0.000
P42	13	0.565	390.215
P43	13	0.196	51.496
P44	4	0.471	303.929
P45	3	0.092	0.000
P46	13	0.120	0.000
P47	13	0.199	54.250
P48	13	2.347	2025.978
P49	4	1.070	0.000
P50	8	0.097	0.000
P51	10	0.129	0.000
P52	10	0.148	7.435
P53	13	0.096	0.000
P54	13	0.238	90.050
P55	7	0.124	0.000
mean	8.3	0.217	81.603
SD	4.0	0.345	290.320

Table 12. The severity, skin test results, optical density and concentration of anti house dust mite antibody specific to Blo t 1 in AR patients.

No.	Severity score	OD	ng/ml
P1	14	0.134	0.000
P2	10	0.095	0.000
P3	4	0.101	0.000
P4	8	0.110	0.000
P5	4	0.100	0.000
P6	13	0.117	0.000
P7	9	0.100	0.000
P8	5	0.101	0.000
P9	13	0.125	0.000
P10	4	0.096	0.000
P11	3	0.089	0.000
P12	13	0.087	0.000
P13	8	0.097	0.000
P14	11	0.096	0.000
P15	4	0.135	0.000
P16	8	0.121	0.000
P17	13	0.135	0.000
P18	7	0.107	0.000
P19	4	0.109	0.000
P20	8	0.103	0.000
P21	4	0.093	0.000
P22	4	0.101	0.000
P23	13	0.089	0.000
P24	6	0.097	0.000
P25	3	0.110	0.000
P26	4	0.097	0.000
P27	4	0.129	0.000
P28	13	0.134	0.000
P29	4	0.088	0.000
P30	3	0.094	0.000

Table 12. The severity, skin test results, optical density and concentration of anti house dust mite antibody specific to Blo t 1 in AR patients (continued).

No.	Severity score	OD	ng/ml
P31	14	0.096	0.000
P32	3	0.102	0.000
P33	3	0.094	0.000
P34	7	0.130	0.000
P35	6	0.091	0.000
P36	4	0.091	0.000
P37	13	0.135	0.000
P38	13	0.121	0.000
P39	11	0.101	0.000
P40	10	0.115	0.000
P41	13	0.144	3.764
P42	13	0.105	0.000
P43	13	0.110	0.000
P44	4	0.103	0.000
P45	3	0.098	0.000
P46	13	0.094	0.000
P47	13	0.098	0.000
P48	13	0.126	0.000
P49	4	0.141	1.010
P50	8	0.118	0.000
P51	10	0.147	6.517
P52	10	0.127	0.000
P53	13	0.098	0.000
P54	13	0.128	0.000
P55	7	0.091	0.000
mean	8.3	0.109	0.205
SD	4.0	0.017	1.012

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ABSTRACTS AND PRESENTATIONS

Yuwatida S, Supranee F, Sumalee P, and Volaluck S. Mast cell and bacteria in nasal cavity of allergic rhinitis patients. *Oral presentation* at the 20th National Congress on Allergy and Immunology, Bangkok, Thailand, April 29-30, 2004.

Yuwatida Sangrut, Supranee Fooanant, Sumalee Pruksakorn, Volaluck Supajatura. Connective tissue mast cell migration is correlated with the severity of allergic rhinitis. *Poster presentation* at the 28th Annual Scientific Meeting on Mahidol's day, Chiang Mai, Thailand, September 24, 2004.