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APPENDIX 1

The major rubber producer of the world and domestically used in each country (metric ton).

Year	Malasia	Domestic used	Indonesia	Domestic used	Thailand	Domestic used	World
1990	1291000	203500	1262000	173000	1275300	144100	5120000
1991	1255700	237000	1284000	177000	1341200	148700	5170000
1992	1173200	284600	1387000	200000	1531000	178400	5460000
1993	1074300	317600	1301300	212000	1553400	205200	5340000
1994	1100600	343200	1360800	224000	1717900	207200	5670000
1995	1089300	392400	1466800	245000	1784400	229000	5880000

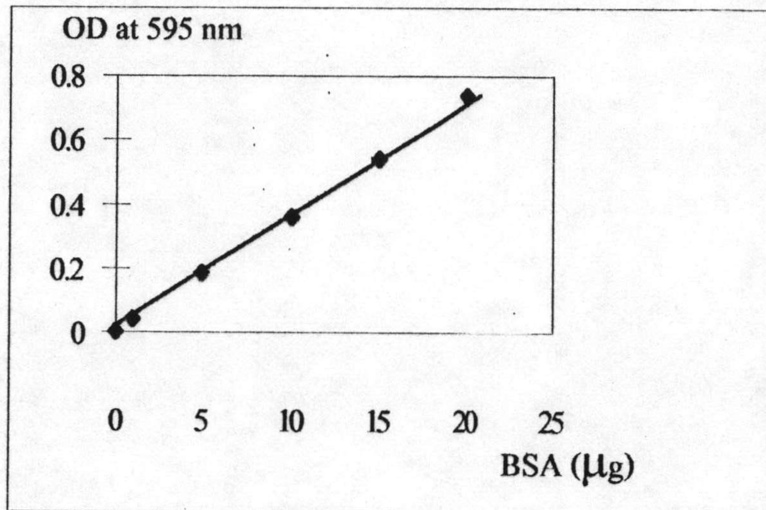
From: IRSG Rubber statistical bulletin vol. 51 no.4 Jan. 1997, Wembley, UK: Redwood Books, 1997.

APPENDIX 2

Protein determination-Bradford's method

Coomassie brilliant blue G 100 mg was dissolved in 50 ml 95 % ethanol. This solution was mixed with 100 ml of 85% w/v phosphoric acid, and then diluted with distilled water to the final volume of 1 l and filtered.

This reagent 1.0 ml was added to 100 μl of sample solution and mixed either by vortexing or several inversion and measured for absorbance at 595 nm after 2-15 min. Standard 0.1 mg/ml protein solution (BSA) have been used every assay.



Standard curve of BSA measured by Bradford (1976).

APPENDIX 3

Solution for SDS-PAGE.

1. Tris glycine electrode buffer (1 l)

(0.025 M Tris, 0.192 M glycine)

Tris	3.03	g
glycine	14.4	g
SDS	5.0	g
H_2O	1	l

2. Tris SDS stock solution pH 6.8

(0.25 M Tris)

Tris	3.94	g
SDS	0.2	g
H_2O	100	ml

(adjust pH to 6.8 with 0.1 M HCl or 0.1 NaOH)

3. Tris-SDS stock solution pH 8.8

Tris	11.82	g
SDS	0.2	g
H_2O	100	ml

(adjust pH to 6.8 with 0.1 M HCl or 0.1 NaOH)

4. Sample buffer

Tris-SDS stock solution pH 6.8	25	ml
SDS	2	g
glycerol	10	ml
2-mercaptoethanol	5	ml
1% bromphenol blue	0.1	ml
H_2O until	100	ml

5. Acrylamide stock (30 %)

Acrylamide	30	g
Bis	0.8	g

6. Ammonium persulfate 0.1 g/ml

7. 15 % Separating gel

Stock gel (30%)	5	ml
Stock buffer pH 8.8	2.5	ml
H_2O	2.5	ml
Amm.persulfate	50	μl
TEMMEP	5	μl

8. Stacking gel

Stock gel (30%)	0.67	ml
Stock buffer pH 6.8	1.0	ml
H_2O	2.3	ml
Ammonium persulfate	30	μl
TEMMEP	5	μl

9. Staining solution

Coomasei blue R250	0.1	g
methanol	45	ml
gacial acetic acid	10	ml
H_2O	45	ml

10. Destain solution

gacial acetic acid	100	ml
methanol	100	ml
H_2O	800	ml

11. **Diethanolamine buffer** (pH 9.5)

(for 0.1 % p-nitrophenyl phosphate)

Diethanolamine	10	mM
$MgCl_2$	0.5	mM

(From: Manson, M. M. Immuno chemical Protocols : Method in molecular biology vol.10 . New York : Robert E. Krieger, 1985.)

APPENDIX 4

Protein blotting

The following protocol has been modified from Gershoni (1984) :-

1. **Materials**

a. Transfer buffer: 15.6 mM Tris, 120 mM glycine, approximate pH = 8.3.

b. PBS, phosphate buffered saline: 10 mM phosphate buffer (pH 7.4) : 150 mM NaCl.

c. Bovine serum albumin.

d. Sodium azide.

e. Bio-Rad Transblot apparatus.

f. SDS-polyacrylamide slab gel.

g. Nitrocellulose membrane.

2. **Electrophoretic blotting**

a. Prepare transfer buffer 24 hrs before blotting and store at 4°C. The volume prepared should be enough to fill the transfer apparatus and wet the immobilizing nitrocellulose (approximately 4 liters).

b. Protein of interest is electrophoresed on an SDS-polyacrylamide slab gel using previously established conditions.

c. Just before the end of the run, pour the cold transfer buffer into the electrophoretic transfer apparatus so as to thoroughly wet the supportive Scotch Brite pads.

d. Cut pieces of immobilizing nitrocellulose membrane to the size of gel(s) to be blotted and wet by floating the membrane on the surface of the transfer buffer. It is important to wet the membrane in this manner so that trapped air is expelled as buffer is drawn into it.

e. Once the dye front of the gel has reached the desired destination the gel should be removed from between the glass plates. The stacking gel should be removed and thus not used for transfer as it is very sticky and difficult to remove from the final blot.

f. The gel is then placed on a pre-wet Scotch Brite pad. This can be accomplished by putting a wet Scotch Brite pad in a large dish (glass or plastic), then placing the gel to be blotted on the pad.

g. Rinse the surface of the gel with transfer buffer avoiding formation of bubbles. (This rinse serves to remove small gel particles adhering to the surface, and also provides a wet surface for the immobilizing matrix, of appropiate size, on the gel. Take care not to trap air bubbles between the gel and the nitrocellulose membrane to adhere.)

h. Apply a wet piece of immobilizing nitrocellulose, of appropiate size, on the gel. Take care not to trap air bubbles between the gel and the membrane.

i. A second Scotch Brite pad is then placed on the membrane, (the gel-membrane composite can sandwiched between two sheets of blotting paper to protect it from nicks and scratches that can be caused by the Scotch Brite pads). This assembly should be well supported between plastic grids and held together snugly (thus providing good continuous contact between the gel and the membrane and placed into the buffer-filled transfer apparatus). The membrane should face the anode side of the transfer apparatus when anionic macromolecules are being transferred.

j. transfer should continue for approximately two hours at 200 mA constant current. Under these conditions, the voltage drops as the transfer progresses due to an increase in the conductivity of the buffer (caused by elution of electrolytes from the gel). If constant voltage is maintained in stead of constant current, the current increases during the transfer and thus a power supply which can cope with this anticipated increase should be used.

k. At the end of the transfer, remove the gel membrane composite from the apparatus and separate the two. The transferred dye front can be marked on the blot with a pen or pencil. Other information pertinent to the experiment may be written on the membrane filter just beyond the dye front to provide easy identification of the blot. Rinse in transfer buffer to remove any adhering polyacrylamide.

l. The wet membrane can be used immediately or stored dry between blotting paper.

(From: Gershoni, J.M. Protein blotting : Principles and applications. New York Feinberg Graduate School, 1984.)

APPENDIX 5

Determination of dry rubber content (% DRC).

Dry rubber content is the weight of rubber in 100 g of latex. It is determined by weighing about 5 g of fresh latex which was then coagulated with 5% acetic acid in methanol and left overnight. The coagulum was sheeted out by steel rolling pin and thoroughly washed with water. The rubber sheet was then dried in an oven at $60^{\circ}C$ to a constant weight. The % DRC was calculated as formulated in:

$$\%DRC = \frac{\text{w of dry rubber} \times 100}{\text{w of latex}}$$

APPENDIX 6

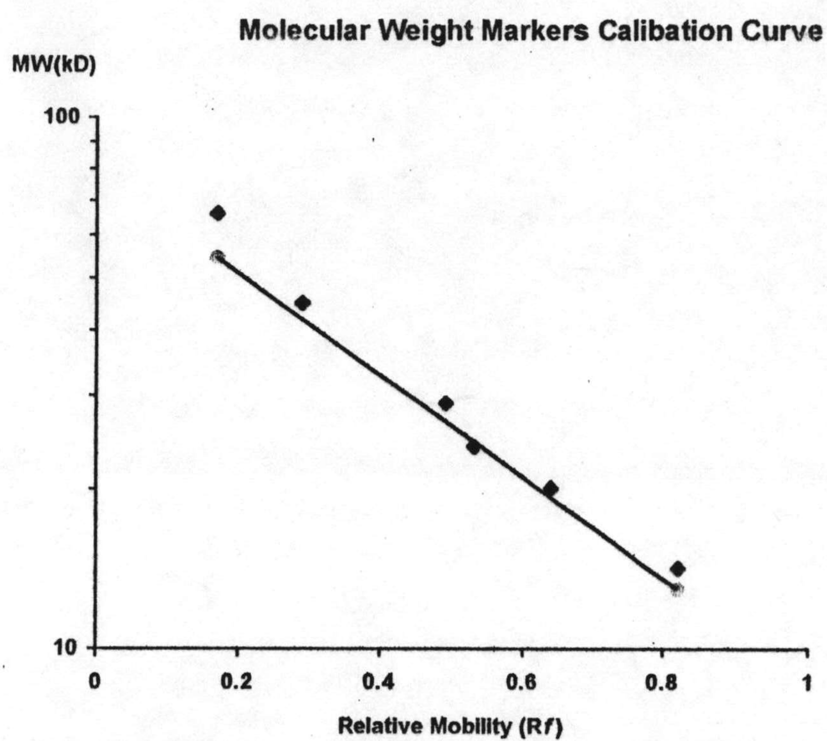


Figure A1 Molecular weight markers calibration curve of SDS-PAGE with regression equation is logY = - 0.96483 X + 1.900406 .

APPENDIX 7

Statistical calculation:

Case-control study fourfold table used for calculation the risk factor in this study and the Chi-square test data calculated from statistical software package (Ecosoft Inc., USA).

	Atopic	Normal	Total
IgE positive	22 (a)	16 (b)	38 (g)
IgE negative	178 (c)	336 (d)	514 (h)
Total	200 (e)	352 (f)	552 (n)

$$X^2 = \frac{(ad-bc)^2 n}{efgh}$$

$$= 8.289$$

$$\text{Relative risk} = \frac{ad}{bc}$$

$$= 2.595$$

----------------------CROSSTAB/CHI-SQUARE TESTS--------------------

	1	2	TOTAL
1	22	16	38
2	178	336	514
TOTAL	200	352	552

CHI-SQUARE WITH CONTINUITY CORRECTION FACTOR = 7.313, PROB.=6.847×10^{-3}

CHI-SQUARE WITHOUT CONTINUITY CORRECTION FACTOR = 8.289, PROB.= 3.989×10^{-3}

D.F. = 1

FISHER EXACT PROBABILITY: Lower Tail, Upper Tail = 3.998×10^{-3}

HYPOTHESIS TEST FOR TWO PROPORTION FROM INDEPENDENT GROUPS

P1 = .1081, N1 = 37

P2 = .1270, N2 = 63

Z = -0.280 PROB.= 0.3896

HYPOTHESIS TEST FOR TWO PROPORTION FROM INDEPENDENT GROUPS

P1 = .1071, N1 = 56

P2 = .0909, N2 = 44

Z = -0.269 PROB.= 0.3941

HYPOTHESIS TEST FOR TWO PROPORTION FROM INDEPENDENT GROUPS

P1 = .0398, N1 = 176

P2 = .0511, N2 = 176

Z = -0.512 PROB.= 0.3044

HYPOTHESIS TEST FOR TWO PROPORTION FROM INDEPENDENT GROUPS

P1 = .1200, N1 = 100

P2 = .1000, N2 = 100

Z = -0.452 PROB.= 0.3256

APPENDIX 8

Per cent inhibition of indirect ELISA with added allergen (Figure 3.8).

Allergen added (μg)	0.003125	0.00625	0.0125	0.025	0.05	0.1	0.5	1.0
% inhibition	2.20	4.41	8.82	17.73	35.70	70.20	96.90	99.10

Per cent inhibition of latex proteins, gloves or tires extracted proteins (Figure 3.9-10).

Protein added (μg)	0.05	0.1	0.5	1.0	5.0	10.0
% inhibition of						
RRIM600	5.2	13.6	45.8	72.5	97.2	98.8
GT1	4.7	10.6	40.7	72.6	97.9	99.2
PB5/51	5.1	12.6	47.2	77.6	96.6	98.8
KRS165	4.9	12.4	52.6	78.1	96.9	99.5
Gloves (mean)	2.2	10.1	32.6	51.4	85.5	95.3
Glove A	2.3	12.5	33.4	53.1	88.5	98.5
Glove B	2.3	10.6	32.8	51.3	85.0	95.3
Glove C	2.0	7..2	31.6	49.8	83.0	92.1
Tires (mean)	1.1	4.3	14.9	35.1	74.6	90.8
Firestone	1.2	4.4	15.5	36.4	76.7	95.6
Bridgestone	1.1	4.5	15.3	35.6	75.4	93.4
Michelin	1.3	4.3	14.8	34.7	74.3	90.1
Siam Tire	0.8	4.1	14.0	33.7	72.0	84.1

BIOGRAPHY

Lieutenant Kitiphong Harncharoen was born on December 27, 1958 in Bangkok. He graduated with the degree of Bachelor of Science (Med.Tech.) from Khon Kaen University in 1984 and Master of Science (Trop.Med.) from Mahidol University in 1990. He had worked in the Microbiology Department, Ramathibodi Hospital, Faculty of Medicine, Mahidol University in a position of Medical Technologist from 1984 to 1985. In 1985-1986, He had worked in Prapinklao, Naval Hospital and had been a guest lecturer in Microbiology at Naval Nursing College. He had worked in a position of an Acting Chief Medical Technologist Biochemistry Division, Arbhakornkeatiworng, Naval Base Hospital from 1986 to 1988. In 1988-1990, he had worked in the position of an Acting Researcher, Technical Division, Naval Medical Department, after that he had worked in the position of a Head of Microbiology Division, Prapinklao Hospital and had been a guest lecturer in Microbiology, Immunology, Parasitology and Biochemistry at Naval Nursing Collage until 1993.