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APPENDICES

APPENDICES

APPENDIX A

Aluminium content assay

Table 16 The percentage of aluminium content in aluminium hydroxide gel.

Adjuvant	% Aluminium	Av. % aluminium (± SD)
Aluminium hydroxide gel	2.27 2.26 2.25	2.26 ± 0.01

APPENDIX B

The concentration unit of DT, TT and JE

Table 17 The calculation of concentration unit between µg/ml with Lf/ml or antigen unit/ml of diphtheria toxoid, tetanus toxoid and JE antigen.

Antigen	conc. (µg/ml) from BCA					Av. Conc. (µg/ml)	%CV	conc. (Lf/ml)	content / Lf
DT	1201.15	1105.28	1179.49	1034.87	1194.36	1198.13	8.39	300.00	3.99
	1351.79	1087.56	1262.67	1305.47	1258.69				
TT	297.52	339.64	309.26	313.91	321.15	312.80	6.20	60.00	5.21
	327.86	282.58	315.87	334.43	285.77				

Antigen	conc. (µg/ml) from BCA					Av. Conc. (µg/ml)	%CV	conc. (antigen unit/ml)	content/ antigen unit
JE	1476.33	1606.39	1541.83	1210.48	1307.56	1424.96	10.05	8.80	161.93
	1625.12	1351.74	1260.68	1472.62	1396.87				

Adsorptive capacity of single antigen on adjuvant

Table 18 Adsorption of diphtheria toxoid on aluminium hydroxide.

conc. DT (µg/ml)	Average adsorption (mg antigen / mg Al)	SD
179.72	0.22	0.01
359.44	0.42	0.01
479.25	0.41	0.01
599.07	0.39	0.00
718.88	0.39	0.01
838.69	0.41	0.01
898.60	0.42	0.00
958.51	0.46	0.00
1018.41	0.45	0.01
1078.32	0.45	0.01
1138.23	0.46	0.01

Table 19 Adsorption of tetanus toxoid on aluminium hydroxide.

conc. TT (µg/ml)	Average adsorption (mg antigen / mg Al)	SD
31.28	0.08	0.01
62.56	0.16	0.00
93.84	0.24	0.01
125.12	0.33	0.00
156.40	0.41	0.01
187.68	0.46	0.06
218.96	0.51	0.04
250.24	0.54	0.00
281.52	0.60	0.02
297.16	0.60	0.09

Table 20 Adsorption of JE antigen on aluminium hydroxide.

conc. JE (μg/ml)	Average adsorption (mg antigen / mg Al)	SD
71.25	0.04	0.00
142.50	0.06	0.01
284.99	0.13	0.00
427.49	0.11	0.01
569.99	0.14	0.03
712.48	0.09	0.02
854.98	0.12	0.00
997.47	0.30	0.00
1139.97	0.43	0.00
1282.47	0.24	0.01

APPENDIX C

ELISA reagent

1. 0.05 M Carbonate-bicarbonate buffer (pH 9.6) (coating buffer)

Sodium carbonate	0.8	g.
Sodium hydrogen carbonate	1.5	g.
Distrilled water to	500	ml.

(adjust pH to 9.6 before bringing to volume)

2. Phosphate buffer saline (PBS) pH 7.4 with 0.05% Tween 20 (PBS-T , washing buffer)

Sodium chloride	8.0	g.
Potassium dihydrogen phosphate	0.2	g.
Disodium hydrogen phosphate	2.9	g.
Potassium chloride	0.2	g.
Thimerosol	0.1	g.
Tween 20	0.5	ml.
Distrilled water to	1,000	ml.

(adjust pH to 7.4 before bringing to volume)

3. 3% gelatin in PBS-T (blocking solution)

gelatin	3.0	g.
PBS-T to	100.0	ml.

4. Citrate – phosphate buffer pH 5.0 (substrate buffer)

Citric acid (monohydrate)	10.30	g.
Sodium hydrogen phosphate ($Na_2HPO_4 .2H_2O$)	18.16	g.
30% Hydrogen peroxide	1.0	ml.
Distrilled water to	1,000	ml.

5. 1% gelatin in PBS-T (diluent)

gelatin	1.0	g.
PBS-T to	100.0	ml.

6. 4 N Sulfuric acid (stop solution)

98% Sulfuric acid	54.4	ml.
Distrilled water to	500.0	ml.

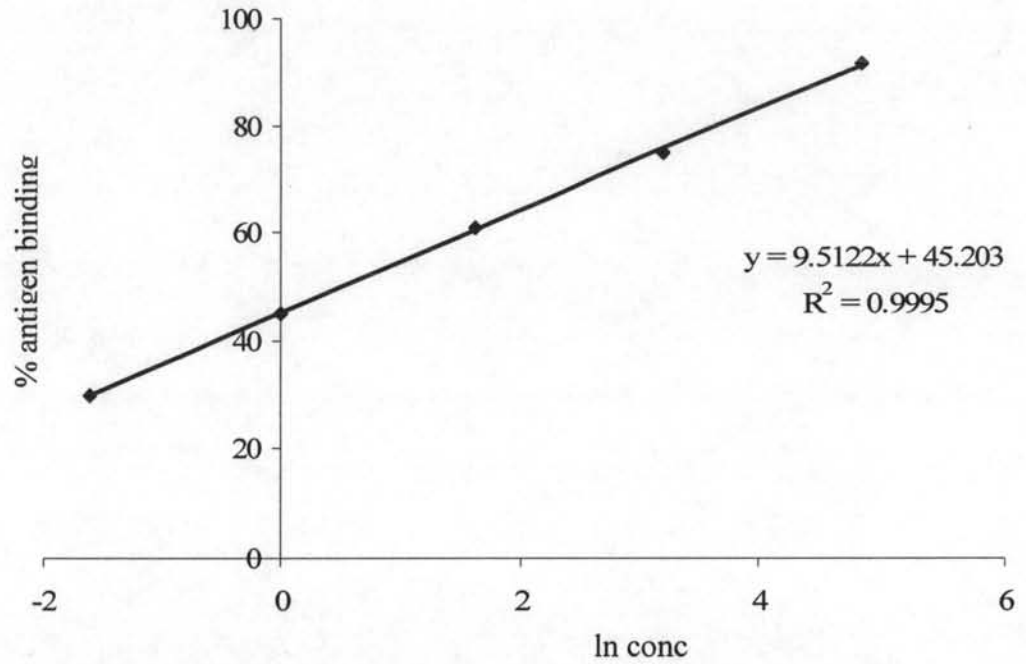

Figure 24 Standard curve of the optimal condition of diphtheria toxoid

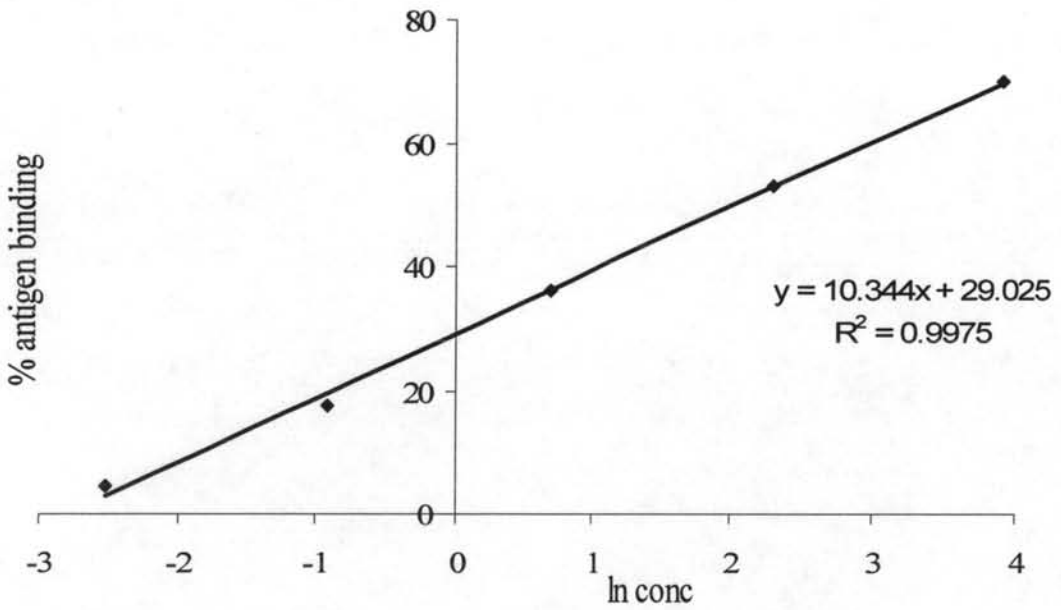

Figure 25 Standard curve of the optimal condition of tetanus toxoid

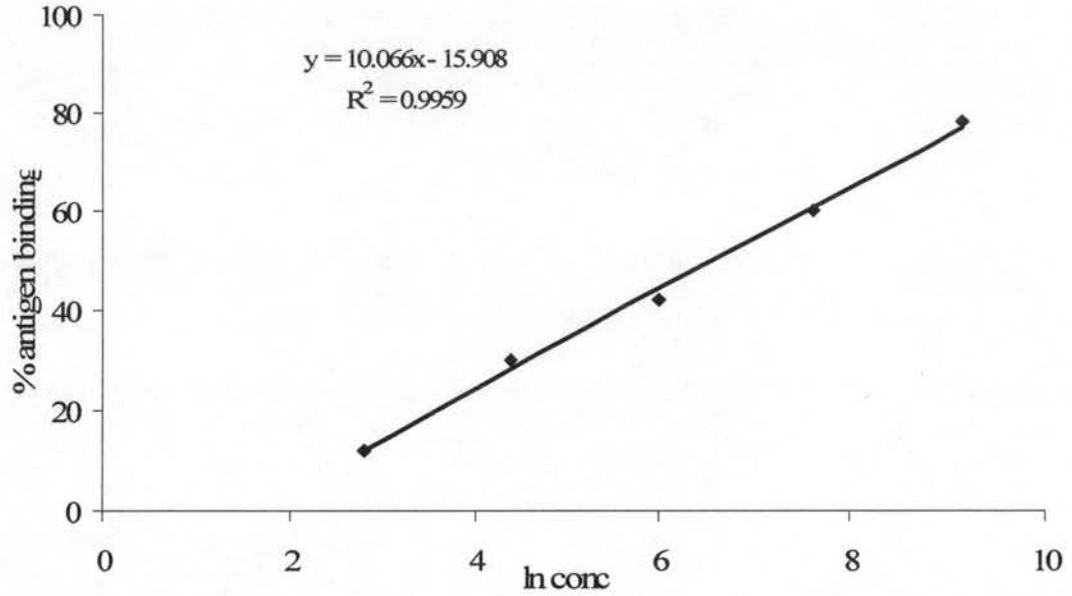

Figure 26 Standard curve of the optimal condition of *Bordetella pertussis*

APPENDIX D

Table 21 Statistical test for the percentage of adsorption of antigens on aluminium hydroxide adjuvant at various temperatures

Analysis of variance (ANOVA) and LSD test for pairwise comparisons

Dependent variable: %adsorption value

LSD

(I) Temperature(°C)	(J) Temperature(°C)	DT	TT	JE
		Sig.	Sig.	Sig.
5	15	.000*	.342	.084
5	25	.000*	.000*	.069
5	37	.000*	.000*	.000*
15	25	.239	.000*	.920
15	37	.000*	.000*	.000*
25	37	.000*	.000*	.001*

*. The mean difference is significant at the .05 level.

Table 22 Statistical test for the percentage of adsorption of antigens on aluminium hydroxide adjuvant at various mixing speed

Analysis of variance (ANOVA) and LSD test for pairwise comparisons

Dependent variable: %adsorption value

LSD

(I) Mixing speed(rpm)	(J) Mixing speed(rpm)	DT	TT	JE
		Sig.	Sig.	Sig.
200	300	.537	.420	.149
200	400	.005*	.001*	.009*
200	500	.957	.001*	.021*
300	400	.001*	.004*	.000*
300	500	.503	.005*	.001*
400	500	.006*	.936	.718

*. The mean difference is significant at the .05 level.

Table 23 Statistical test for the percentage of adsorption of antigens on aluminium hydroxide adjuvant at various mixing time

Analysis of variance (ANOVA) and LSD test for pairwise comparisons

Dependent variable: %adsorption value

LSD

(I) Mixing time (hr)	(J) Mixing time (hr)	DT	TT	JE
		Sig.	Sig.	Sig.
1	5	.001*	.119	.000*
1	12	.077	.131	.676
1	24	.040*	.464	.147
5	12	.048*	.958	.000*
5	24	.000*	.026*	.000*
12	24	.000*	.029*	.066

*. The mean difference is significant at the .05 level.

Table 24 Statistical test for particle size distribution (LD) of AH and combined preparations at initial and after 4-months storage at 2-8 °C

Paired sample t-test

Pairs	Sig.
AH – C0	.001*
AH – S0	.241
C0 – S0	.003*
C0 – C4	.001*
S0 – S4	.001*
C4 – S4	.002*

C0, C4: competitive adsorption at initial, 4-month storage

S0, S4: separate adsorption at initial, 4-month storage

*. The mean difference is significant at the .05 level.

Table 25 Statistical test for the antigen contents among C and S

Analysis of variance (ANOVA) and LSD test for post hoc comparisons

Dependent variable: antigen content value

LSD

(I) formula	(J) formula	DT				
		t_0	t_1	t_2	t_3	t_4
		Sig.	Sig.	Sig.	Sig.	Sig.
C	S	.077	.161	.044*	.011*	.011*

* The mean difference is significant at the .05 level.

(I) formula	(J) formula	TT				
		t_0	t_1	t_2	t_3	t_4
		Sig.	Sig.	Sig.	Sig.	Sig.
C	S	.030*	.022*	.269	.058	.263

* The mean difference is significant at the .05 level.

(I) formula	(J) formula	PT				
		t_0	t_1	t_2	t_3	t_4
		Sig.	Sig.	Sig.	Sig.	Sig.
C	S	.408	.176	.05*	.217	.153

* The mean difference is significant at the .05 level.

(I) formula	(J) formula	JE				
		t_0	t_1	t_2	t_3	t_4
		Sig.	Sig.	Sig.	Sig.	Sig.
C	S	.014*	.094	.043*	.064	.177

* The mean difference is significant at the .05 level.

Table 26 Statistical test for the antigen contents among initial, 1 month, 2 months, 3 months and 4 months

(I) months	(J) months	DT		TT	
		C	S	C	S
		Sig.	Sig.	Sig.	Sig.
Initial	1 month	.004*	.091	.008*	.005*
Initial	2 months	.003*	.027*	.017*	.123
Initial	3 months	.001*	.009*	.002*	.003*
Initial	4 months	.000*	.002*	.002*	.001*
1 month	2 months	.004*	.081	.003*	.031*
1 month	3 months	.001*	.045*	.001*	.002*
1 month	4 months	.001*	.022*	.001*	.000*
2 months	3 months	.001*	.046*	.016*	.277
2 months	4 months	.005*	.030*	.008*	.053
3 months	4 months	.008*	.023*	.001*	.004*

* The mean difference is significant at the .05 level.

(I) months	(J) months	PT		JE	
		C	S	C	S
		Sig.	Sig.	Sig.	Sig.
Initial	1 month	.165	.075	.001*	.003*
Initial	2 months	.015*	.020*	.001*	.001*
Initial	3 months	.002*	.002*	.001*	.001*
Initial	4 months	.001*	.006*	.000*	.001*
1 month	2 months	.010*	.022*	.006*	.006*
1 month	3 months	.009*	.002*	.009*	.002*
1 month	4 months	.013*	.008*	.001*	.000*
2 months	3 months	.010*	.055	.070*	.001*
2 months	4 months	.015*	.007*	.001*	.001*
3 months	4 months	.024*	.057	.020*	.021*

* The mean difference is significant at the .05 level.

All statistic analysis was calculated using SPSS version 13.0.

VITA

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