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APPENDIX I

The Identification of Snake Species

In Bangkok, there are two species of Green pit viper that are *Trimeresurus albolabris* or light green snake and *Trimeresurus macrops* or dark green snake. The differences are (Suebsan Mahasandana et al, 1990) :

1. The Habitat : The ratio between *T. albolabris* and *T. macrops* in Bangkok is 1.7:1 but the ratio is reversed in Thonburi.

2. The severity : *T. albolabris* victims are more likely to have severe envenoming than *T. macrops* but there is no evidence that each venom has different mechanism of action.

3. The morphology criteria (differentiation of the two species in this study is based on these criteria) :

Characteristics	*T. albolabris*	*T. macrops*
Color of body	Yellowish green	Dark green
Color of underside	Yellow/white	bluish green
Color of tail	Reddish	Reddish brown
Eye	Round, small	Cat-like eye round , big

Characteristics	*T. albolabris*	*T. macrops*
Labial	Yellow/white	Bluish-green
Head	slimmer	broader and shorter
Supraocular scales	slimmer	broader
Internasal scales	smaller	bigger

APPENDIX II

Specimen Collection

Blood samples are drawn on the first day the patients come to snake bite clinic. Most are in the morning about 9.00 AM. Most sample will be collected from 9:00 AM to 11:30 AM). For IPD cases, the specimen will be taken as soon as possible after admission. After at least 5-minute rest, the blood will be drawn as soon as possible after applying the tourniquet to prevent venous stasis. Tourniquet application will not be lasted more than 2 minutes. If the vein cannot be found within 2 minutes, the tourniquet will be brought out for a while and reapplied. The double-syringe technique is used. The first 2-3 ml of blood drawn by the first plastic syringe is sent for complete blood count and another plastic syringe will be used to draw more specimen for coagulation/fibrinolysis study. The samples are immediately anticoagulated. Fibrinopeptide A assay requires special anticoagulant prepared in the test kit. The 3.8% sodium citrate is used for the other tests. The ratio for volume of blood and anticoagulant is exactly 9:1. The specimen is centrifuged immediately at 3,000 rpm in the temperature range of $+10^{o}$ and $+18^{o}$ for 15 minutes. The plasma is collected and then immediately stored at -80^{o} until test.

Normal Values

APTT	not more than 5 seconds over control
PT	not more than 3 seconds over control
TT	not more than 3 seconds over control
Fibrinogen	200 - 400 mg/dl
ELT	more than 120 minutes
plasminogen	88 - 139 % activity (mean $\pm$ 2SD of control)
antiplasmin	82 - 130% activity (mean $\pm$ 2SD of control)

APPENDIX III

Variations of Fibrinolytic Parameters and Their Corrections

1. Age : The fibrinolytic parameters are varied between age. Fibrinolytic activity is decreasing with age due to plasminogen activator inhibitor (PAI) elevation (Krishnamurti et al, 1988). The controls will be selected by their age (to be in the same age group as the patients).

2. Sex : Female subjects have higher fibrinolytic activity than male subjects because of higher t-PA and lower PAI (Koh et al, 1991).

3. Diurnal variation : Fibrinolytic activity is lowest in the morning and hishest in the evening because of the fluctuation of PAI levels (Bachman, 1994). Most outpatients have blood drawn in the morning about 9.00 AM. However, for inpatient, we cannot control the time of blood taken because urgent antivenin is required. The delayed specimen collection will caused more biases than immediate collection (Booth, 1991). The parameters of patients will be changed with time and therapy but diurnal variation is usually subtle and not clinically significant. The control group will have blood taken at about 9.00AM to match most of the patients. The time of sample collection will be recorded.

4. Diet : Some kinds of food effect fibrinolysis such as onion and garlics increase fibrinolytic activity (Bachman, 1994). The ideal specimen is from overnight-fast subjects. But most cases that come to the hospital do not fast. The delay of measurements will be much more inaccurate than immediate collection (Booth, 1991). Therefore, the fasting is usually omitted in the setting of acute disease such as snake bite.

5. Alcohol (Veenstra et al, 1990), caffeine (Booth, 1991) and smoking (Allen, Kluft and Brommer, 1984 and 1985): Both have some effects but the abstinence before blood taken is not necessary because it may result in selection bias (Booth, 1991). The history of recent alcohol, caffeine containing beverage and smoking will be recorded.

6. Physical activity and exercise : Vigorous exercise temporary activates fibrinolysis (Defaux, Order and Liesen, 1991). At least 5-minute rest is usually sufficient (Booth, 1991).

7. Pregnancy : Pregnancy causes hypofibrinolysis due to PAI elevation (Bonnar, Daly and Sheppard, 1990). The pregnant women will be excluded from the study.

8. Underlying diseases and drugs : The cases with these conditions will be excluded according to exclusion criteria. Drugs that affect fibrinolysis are listed below.

Anabolic steroid (Kluft et al, 1984)
Antidiabetic drugs (Bachman, 1994)
Aspirin 900-1500 mg/day (Bachman, 1994)
Angiotensin Converting Enzyme Inhibitor (Wright et al, 1994)
Bezafibrate (Bachman, 1994)
Benzodiazepine (Bachman, 1994)
β_2 agonist (Bachman, 1994)
Furosemide (Bachman, 1994)
Gemfibrozil (Fujii and Sobel, 1992)
Heparin (Bachman, 1994)
Lovastatin (Isaacsohn et al, 1994)
Oral contraceptive pills (Kooistra at al, 1990)

9. Venous stasis : Prolonged tourniquet application stimulate t-PA release. In our study, we try to avoid venous stasis as much as possible.

10. Specimen Processing :

Platelet Contamination : The preferable specimen is platelet-poor plasma. Therefore, high-speed centrifugation is required. Samples will be processed immediately after drawn and centrifuged in the cold temperature to prevent platelet activation. Plasmin generated by platelet may caused fibrinolytic activation *in vitro*.

For fibrinopeptide A assay, specific protease inhibitor is required to stop the process of FpA generation *in vitro*. Therefore, two tubes of blood are used. One is for FpA that requires special anticoagulant. One for the others that require citrated plasma.

The ratio between blood and anticoagulant must be accurate. Too much or too little blood will give the false values.

The samples kept in -80^{o} can be used for as much as a year.