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APPENDIX

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APPENDIX

1. Equipments

1.1 Air Jacket CO_2 Incubator (NuAire: model NU-8700)

1.2 Analytical balance (Precia: model XB2200C)

1.3 Analytical balance (Ohaus: model AR2140)

1.4 Autoclave (Hiclave: model HVA-85)

1.5 Centrifuge (Beckman Coulter: model Allegra X-15R)

1.6 Flow cytometry (BD Biosciences: model FACS calibur)

1.7 Fluorescent /Phase contrast microscope (Olympus: model DP-72)

1.8 Freezer-20°C (Sanyo)

1.9 Freezer–80°C (Sanyo: model MDF-U5086W)

1.10 Hot air oven (Binder: model B28)

1.11 Hot plate stirrer (LabTech®: model LMS-100)

1.12 Inverted microscope (Nikon: model TS100)

1.13 Laminar flow (Thermo electron corporation: model SAFE 2010)

1.14 Micro fluid Spectrophotometer (Thermo Scientific: model NanoDrop 1000)

1.15 Microtome (Shadon Finesse)

1.16 Oven (Binder: model B28)

1.17 pH meter (Fisher scientific: model AB15)

1.18 Refrigerator 4°C (Sharp: model SJD56N)

1.19 Refrigerated Centrifuge (Boeco: model U32R)

1.20 Refrigerate microcentrifuge (Eppendorf: model 5415R)

1.21 Spectrophotometer (Thermo Fisher Scientific: model Multiskan EX)

1.22 Tissue embedding (LEICA: model EG1150H)

1.23 Tissue processor

1.24 Vaccuum pump

1.25 Vortex mixer (Labnet: model VX100)

1.26 Water bath (Memmert: model M22)

2. Materials

1.27 Adhesive cap 500 opaque (Carl Zeiss)

1.28 Adjustable micropipette: P2 (0.1-2 µl), P10 (0.5-10 µl), P20 (5-20 µl), P100 (20-100 µl), P1000 (100-1,000 µl) (Gilson)

1.29 Beaker: 50 ml, 100 ml, 200 ml, 500ml, 1000 ml (Pyrex)

1.30 Coplin jar

1.31 Cylinder: 25 ml, 50 ml, 100 ml, 250 ml, 500 ml, 1,000 ml (Pyrex)

1.32 Filter paper No.2 (Whatman)

1.33 Flask: 250 ml, 500 ml, 1000ml (Pyrex)

1.34 Glass vaccuum filtration unit (Gibco)

1.35 Lris scissors

1.36 Membrane slide 1.0 PEN (Carl Zeiss)

1.37 Microcentrifuge tube: 0.2 ml, 0.5 ml, 1.5 ml (Axygen)

1.38 Parafilm (American Nation Can)

1.39 Petri dish (Corning)

1.40 Pipette (Corning)

1.41 Pipeting aid (Gilson)

1.42 Pipette tip: 10 µl, 20 µl, 200 µl, 1,000 µl (Axygen)

1.43 Polypropylene conical tube:15 ml และ 50 ml (Corning)

1.44 Reagent bottle: 100 ml, 250 ml, 500 ml, 1000 ml (Pyrex)

1.45 Staining jar

1.46 Stirring-magnetic bar

1.47 Syringe: 5 ml, 10 ml, 20 ml (Thermo)

1.48 Syringe filter: 0.2 µm (pore size) diameter 40 mm (Minisart)

1.49 Tissue culture flask: 25 cm^2 (NUNC)

1.50 Tissue forcep

1.51 Tissue cassette

1.52 Well plate: 6 well plate, 12 well plate (Corning)

3. Chemical

1.53 Cell culture

1) Fetal bovine serum (Gibco: cat.no.10270)
2) Insulin (Sigma-Aldrich: cat.no.I1882)
3) Penicillin-streptomycin 10,000 U/ml (Gibco: cat.no. 15140-122)
4) RPMI medium 1640 (Gibco: cat.no. 31800-022)
5) HAM-F12 culture medium (Gibco Invitrogen, Grand Island, NY, USA)
6) Sodium Bicarbonate (Sigma-Aldrich: cat.no. S4772)
7) Trasferrin (Sigma-Aldrich: cat.no. T0665)
8) Trypsin (Sigma-Aldrich: cat.no. T7409)
9) Collagen I (Gibco: cat.no. IC2931A)
10) Water bath treatment (Andwino Scientific: cat.no. 190009)

1.54 Reagents and chemical for Flow Cytometry

1) Paraformaldehyde
2) Saponin (Sigma-Aldrich: cat.no. S7900)
3) Annexin V/PI kit
4) TUNNEL assay

1.55 Chemical for cell injury studies

1) LDH detection kit (CytoTox 96, Promega, and Madison, WI, USA)

1.56 Chemical for pathology examination

1) PARAPLAST* tissue embedding medium (Mccormick™ scientific)
2) Xylene
3) Ethanol 99.9% (Merck)
4) Deionized water (dH_2O)
5) Tris Buffered Saline (TBS)
6) Citrate

7) EDTA

1.57 General chemical

1) Hydrochloric acid solution (HCl)
2) Phosphate buffer saline (PBS)
3) Sodium hydroxide (NaOH)
4) Sterile water (ready for use)

4. Reagent preparation

a. Culture medium

Fetal bovine serum	10	%
Penicillin	50	µg/ml
Streptomycin	50	µg/ml
Insulin	0.1	µg/ml
Transferrin	5	µg/ml
Selenium	50	µg/ml
In HAM-F12		

b. Phosphate Buffer Saline (PBS)

Sodium chloride (NaCl)	0.137	M
Potassium (KCl)	2.7	mM
Potassium dihydrogen phosphate (KH_2PO_4)	1.4	mM
disodium hydrogen phosphate ($NaHPO_4$)	0.01	M

c. Trypsin-EDTA solution

Trypsin	0.125	% (w/v)
EDTA	0.53	mM
in PBS		

d. 0.5% Periodic Acid Solution

Periodic acid	0.5 g
In water	100 ml

e. Test for Schiff reagent

Pour 37% formalin 10 ml into a watch glass then drop the reagent, a good Schiff reagent will rapidly turn a red- Purple color

4. Periodic acid-Schiff (PAS) staining procedure

1. Deparaffinize and hydrate to water
2. Oxidize in 0.5% periodic acid solution for 5 minutes
3. Rinse in distilled water
4. Place in Schiff reagent for 15 minutes (Section turn light pink color)
5. Wash in lukewarm tap water for 5 minutes (Immediately sections turn dark pink color)
6. Counterstain in Mayer's hematoxylin for 1 minute
7. Dehydrate and coverslip using a sy thetic mounting medium

Pathology examination scoring system

Histological tissue was examined. The absence of a numeral indicates that the finding specified was not identified. Significance of differences in a pair wise (Fisher's) test between Group 1 and each treatment group: * P<0.05, *** P<0.01, *** P<0.001

Version I	Version II
1. Liver	
No abnormality detected Solitary pale cell focus(i) Vacuolation Focal inflammation PAS STAIN: negative PAS STAIN: positive (grade +/-) PAS STAIN: positive (grade +) PAS STAIN: positive (grade ++) Total incidence for score expanded DIASTASE + PAS STAIN: negative DIASTASE + PAS STAIN: positive (kupffer cells, grade +/-) DIASTASE + PAS STAIN: positive (kupffer cells, grade +) DIASTASE + PAS STAIN: positive (kupffer cells, grade ++) Total incidence for score expanded finding	Congestion Vacuolar degeneration Necrosis Cellular infiltration Thrombosis and vasculitis hyperplasia Fibrosis **Key to scores:** - = No lesions observed; ± = Mild, Absent or focal lesions; + = Moderate, multifocal lesions; ++ = Moderately severe, diffuse lesions; +++ = Very severe, diffuse lesions
2. HEART :	

No abnormality detected (Grade +/-) (Grade +)	Congestion / Haemorrhage/ Myofibril Vacuolar degeneration & necrosis Hyalinised vessels, Thrombosis and vasculitis **Key to scores:** - = No lesions observed; ± = Mild, Absent or focal lesions; + = Moderate, multifocal lesions; ++ = Moderately severe, diffuse lesions;
3. KIDNEY :	
No abnormality detected Capsular inflammation Area(s) of inflammation Area(s) of haemorrhage(s) Basophilic tubules Unilateral pelvic dilatation Mineral deposit(s)	0 = Normal structure 1 = No cellular proliferation or fibrosis in renal glomerulus = No capillary congestion or microthrombus = Swelling and blurry boundary of renal tubular epithelial cell, stegnosis or atresia of lumens = Protein cast and renal interstitial edema 2 = Glomerular capillary congestion = Scattered necrosis in renal tubular epithelial cell = Interstitial edema and inflammatory cell infiltration 2 = 2 + lamellar necrosis of renal tubular epithelial cell
4. SPLEEN :	

No abnormality detected Focal capsular inflammation Area(s) of inflammation and necrosis Increased haemosiderin PAS STAIN: positive (grade +/-) PAS STAIN: positive (grade +) PAS STAIN: positive (grade ++) Total incidence for score expanded finding DIASTASE + PAS STAIN: positive (grade +/-) DIASTASE + PAS STAIN: positive (grade +) Total incidence for score expanded finding	0 = Normal structure 1 = Necrosis in the follicular center was seen; 2 = Blood sinus expansion and arteriolosclerosis 3 = Necrosis in the follicular center, blood sinus expansion and arteriolosclerosis

5. MESENTERIC LYMPH NODE:	
No abnormality detected Congestion Reactive Partly replaced by area of inflammation with giant cells Dilated blood vessels with thrombus formation PAS STAIN: positive (grade +/-) PAS STAIN: positive (grade +) PAS STAIN: positive (grade ++) Total incidence for score expanded finding PAS STAIN: not examined DIASTASE + PAS STAIN: positive (grade +/-) DIASTASE + PAS STAIN: positive (grade +) DIASTASE + PAS STAIN: positive (grade ++) Total incidence for score expended finding DIASTASE + PAS STAIN: not examined	0 = Normal structure 1 = Follicle Germinal center dilated, lymphatic sinus dilated, sinus cell hyperplasia or only lymphatic sinus dilated, sinus cell hyperplasia 2 = Follicle Germinal center dilated, lymphatic sinus dilated, sinus cell hyperplasia, spotty necrosis in mantle zone and Germinal center or only lymphatic sinus dilated, and sinus cell hyperplasia, infiltration of neutrophil, eosinophile granulocyte and plasmocyte 3 = Follicle Germinal center dilated, lymphatic sinus dilated, sinus cell hyperplasia, spotty necrosis in mantle zone and Germinal center, infiltration of neutrophil, eosinophile granulocyte and plasmocyte

6. ILEUM :	
No abnormality detected Submucosal pigment deposit(s)	(1) Mucosa intact (epithelium mucosae, glandular epithelium), no necrosis (2) Mucosa incomplete, focal necrosis (3) Edema in lamina propria, submucous layer and placenta percreta (4) Inflammatory cell infiltration (neutrophilic granulocytes, eosinophile granulocytes, large mononuclear cells) in lamina propria, submucous layer, and placenta percreta. Scoring standards (1) + (3) = 0 score (2)or (4) = 1 score (2) + (3) = 2 scores (2) + (4) = 3 scores

Finding
7. LUNGS:
No abnormality detected
Mild inflammatory changes
Increased alveolar macrophages
Area(s) of haemorrhage(s)
Area(s) of alveolar congestion
Medial hypertrophy
8. ADRENALS:
No abnormality detected
Unilateral focal vacuolation
Unilateral focal pigment deposit(s)
9. THYMUS:
No abnormality detected
10. TESTES:
No abnormality detected
Unilateral tubular atrophy: (Grade +/-), (Grade +++)
Total incidence for score expandede finding
Inflammation with or without giant cells
11. PROSTATE:
No abnormality detected
12. OVARES:
No abnormality detected, Unilateral cyst(s)
13. UTERUS:
No abnormality detected
Dilatation in both horns: (Grade +/-), (Grade +++)
Total incidence for score expanded finding
Area(s) of inflammation with giant cells

14. BRAIN:

No abnormality detected, Cyst(s)

15. SKELETAL MUSCLE:

No abnormality detected

16. PANCREAS:

No abnormality detected

Area(s) of inflammation

17. SALIVARY GLAND:

No abnormality detected

18. SUMMANDIBULAR LYMPM NODE:

No abnormality detected

19. PITUITARY:

No abnormality detected

Cyst(s)

20. SKIN/SUBCUTIS:

No abnormality detected

Focal ulceration with inflammation / necrosis

21. MAMMARY GLANDS:

No abnormality detected

22. URINARY BLADDER:

No abnormality detected

23. EYES:

No abnormality detected

24. OPTIC NERVE:

No abnormality detected

25. TONGUE:

No abnormality detected

26. AORTA:

No abnormality detected

27. THYROIDS:

No abnormality detected

Lymphoid foci

28. PARATHYROIDS:

No abnormality detected

29. TRACHEA:

No abnormality detected

30. OESOPHAGUS:

No abnormality detected

31. STOMACH:

No abnormality detected

Dilated/cystic gland(s)

32. DUODENUM:

No abnormality detected

33. JEJUNUM:

No abnormality detected

34. CAECUM:

No abnormality detected

Submucosal oedema

Area(s) of haemorrhage(s)

35. DIALATED BLOOD VESSELS:

Cystic crypt

Increased haemosider in deposition

inflammatory changes

36. COLON:

No abnormality detected

Area(s) of submucosal inflammation and haemorhage

Increased lymphoid tissue

37. RECTUM:

No abnormality detected or Increased lymphoid tissue

38. SCIATIC NERVE:

No abnormality detected

39. STERNUM / RIB:

No abnormality detected

40. LYMPH NODE(S):

BRONCHIAL: congestion

BRONCHIAL: reactive

41. INJECTION / TREATMENT SITE(S):

Subcutaneous oedema

Area(s) of simple subcutaneous inflammation

Area(s) of subcutaneous inflammation with haemorrhage

Area(s) of inflammation with giant cells Area(s) of subcutaneous inflammation with giant cells and haemorrhage

42. ABDOMEN:

Area(s) of inflammation, necrosis and giant cells

43. MESENTERY:

No abnormality detected

Area(s) of haemorrhage(s), simple inflammation

Area(s) of inflammation with giant cells

Area(s) of inflammation with haemorrhage Lymphoid foci

44. FALLOPIAN TUBES:

No abnormality detected Unilateral inflammatory cell infiltrate

Area(s) of inflammation, necrosis and giant cells

No abnormality detected or Unilateral inflammatory cell infiltrate

45. DIAPHRAGM:

No abnormality detected or

Adhesion(s), Inflammatory changes with or without giant cells

BIOGRAPHY

Phonethipsavanh Nouanthong was born on 05 October 1975, in Vientiane municipality, Lao PDR. I received BSc in Medical Technology at Khone Kean University, Thailand (1998), MSc in Health Science at Chiang Mai University, Thailand (2005), and completed a summer course in Biostatistics in Public Health I & II and Epidemiology of HIV/AIDS, Johns Hopkins Bloomberg School of Public Health, the JHU, Maryland, United State. I obtained MBA in International Business (2008). In 1998, I worked as a Medical Technologist at Lao Red Cross, Blood Transfusion Center. In later year, I was assigned to be an Acting Head of Laboratory and Training Section and was nominated to be a Director Assistant for Quality Assurance of Blood Safety Program (2001-2003). During 1999-2003, I also taught Immunology and Blood Banking at Medical Laboratory School, Laos. I worked a Research Assistant during my Master course (2003-2005) at Research Institute of Health Science, Chiang Mai University. Then I joined the HIV-Netherland Australia Thailand Research Collaboration Center in 2006 to 2008.

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