APPENDIX

1. Harris's hematoxylin and eosin procedure

Solution

- **1% acid alcohol**

Hydrochloric acid	1	ml
70% ethyl alcohol	99	ml

- **Ammonia water**

28% ammonium hydroxide	2	ml
Distilled water	800	ml

- **Saturated lithium carbonate**

Lithium carbonate	1.54	gm
Distilled water	100	ml

- **Eosin Phloxine solution**

Eosin stock solution

Eosin Y, water soluble	1	gm
Distilled water	100	ml

Phloxine stock solution

Phloxine B	1	gm
Distilled water	100	ml

Eosin-Phloxine working solution

Eosin stock solution	100	ml
Phloxine stock solution	10	ml
95% ethyl alcohol	780	ml

glacial acetic acid	4	ml

* The solution is good for approximately 1 week.

Harris' hematoxylin

Hematoxylin	5	gm
100% ethyl alcohol	50	ml
Potassium or ammonium, alum	100	gm
Distilled water	1000	ml
Mercuric oxide, red	2.5	gm

Completely dissolve the alum in the distilled water with the aid of heat and a magnetic stirrer. Shake to dissolve the hematoxylin in the alcohol, at room temperature. Remove the alum and distilled water from the heat. Slowly combine the two solutions. Return combined solutions to the heat. Bring to a boil as rapidly as possible, approximately 1 minute or less. Remove from the heat and slowly add the mercuric oxide. If the mercuric oxide is added too rapidly, the reaction will cause the solution to boil up and out of the flask. Return the solution to the heat unit it becomes a dark purple, remove it from the heat, and plunge it into a sink of cold water to cool. The solution is ready for use. Add 20 ml of glacial acetic acid to intensify the nuclear stain. Always filter before each use.

Procedure

1. Deparaffinize slides and hydrate to distilled water
2. Stain in freshly filtered Harris' hematoxylin for 6 minutes.
3. Wash in running tap water for 5 minutes.

4. Differentiate in 1% acid alcohol, 1 to 2 dips.
5. Wash briefly in tap water.
6. Place in weak ammonia water or saturated lithium carbonate solution until sections are bright blue.
7. Wash thoroughly in running tap water for 10 minutes.
8. Place in 80% ethyl alcohol for 1 to 2 minutes.
9. Counterstain in eosin-phoxine solution for 2 minutes.
10. Dehydrate and clear through 2 changes each of 95% ethyl alcohol, absolute ethyl alcohol, and xylene, 2 minutes each.
11. Mount with mounting medium.

Results

Nuclear	blue
Cytoplasm	pink to red
Most other tissue structures	pink to red

2. Lectin histochemistry technique

Solution

All lectins were purchase from Sigma (U.S.A.) (1mg/bottle)

1. Lectin 20 µg/ml concentration

   Lectin stock solution (40 µg/ml)

Lectin	1	mg
PBS	25	ml

   * Aliquot 1 ml/ampoule and keep as the stock solution at -20°c

   Lectin working solution (20 µg/ml)

Lectin stock solution	1	ml
PBS	1	ml

2. Avidine-Biotin Complex

Reagent A	1	µl
Reagent B	1	µl
PBS	498	µl

   * Prepare the solution at least 30 minutes before use.

3. 3% H_2O_2

4. DAB (stock)

   Dissolve DBA 1 tablet in 20 ml. of PBS

   DAB (working solution)

3% H_2O_2	1	µl
DAB	1	ml

   * Add 3% H_2O_2 just before performing the reaction.

Procedure

1. Deparaffin in xylene and hydrate with ethanol series.
2. Block endogenous peroxidase in tissu by incubation with 3% H_2O_2 for 60 minutes.
3. Wash in PBS three changes, then incubate the sections with lectin overnight.
4. Wash in PBS three changes, then incubate the sections with avidin-biotin-peroxidase complex (ABC) for 90 minutes, and then washed again with PBS.
5. Chrome with diaminobenzidine (DAB) in the presence of the substrate H_2O_2 for 1 minute, and wash in PBS.

11. Dehydrate slides in graded alcohols, cleare in xylene and mount with Permount.

Result

The glycoconjugates, which is specifics to each lectin are colored brown.

3. Golgi apparatus staining technique (McDonald's modification of Lascano's technique)

Solution

Fixative

aminoacetic acid (glycine)	1.7 g
distilled water	85 ml
40% formalin	15 ml
concentrated nitric acid	0.5 ml

Reducer

1.5% hydroquinone in 15% formalin.

Procedure

1. Take thin pieces of fresh tissue and place in fixative.
2. Wash in 2 changes of distilled water for a few seconds.
3. Treat with 1.5% aqueous silver nitrate for 4 hours
4. Wash in distilled water for a few seconds.
5. Reduce for $1^1/_2$-2 hours with frequent agitation.
6. Wash in several change of distilled water for 5-10 minutes.
7. Paraffin process, cut thin sections and take down to water, and picking up section on glass slides.

12. Dehydrate slides in graded alcohols, clear in xylene and mount with Permount.

Result

Golgi apparatus	black

Curriculum Vitae

Name	Miss Ransiyakorn Lertlam
Date of Birth	September 23, 1977.
Place of Birth	Nakhonratsima
Education	
March, 1995	Certificate of Mathayom 6, Suranareewitaya School, Nakhonratsima.
March, 1999	Bachelor of Science (Radiology), Faculty of Medical Technology, Mahidol University, Bangkok.
Experience	Radiology technologist, MRI and CT scan Diagnosis Center Chonburi, 1999-2000.