Research process by TMA

2011. 04. .

UNITMA

Ⅰ. Concept of pathological inspection

1. What's Pathology?

- Pathology : a terminology originated from pathos(disease) and logos(study or science)
- The study of the way diseases and illness develop

2. Kinds of pathological inspection

- Hematoxylin & Eosin stain
- Special stain
- Immunohistochemistry (IHC)
- Immunoflorescence
- in situ hybridization (ISH)
- Molecular pathology (Molecular work)
- Cytology
- Electronic microscopes

Ⅱ. Sample tissue & Slide production

1. Biopsy & Autopsy

(1) Biopsy

A. excision: surgical removal of focus with a surgical knife

ex) surface of skin, mucous membrane, lymph knot

B. Scraping or Curetting: curettement or scraping of a growth from a cavity with a curette

ex) concealed part of womb, intestines

C. Punch biopsy : extraction of specimen fragment

ex) skin, cervical region, oral cavity, larynx, bronchus, gullet

D. Needle biopsy : extraction of specimen with puncture in live organs

ex)liver, kidney, lymph knot, prostate, marrow, thyroid gland

(2) Autopsy

An autopsy is an medical examination of a dead body (of adult, infant or baby born dead) by a doctor under a pathology specialist's guidance and control in order to find the cause of a death and to identify etiological factors. It is important medical examination, especially in case of an autopsy of baby born dead, because it can prevent curable disease during next impregnation and hereditary ailments by finding out the cause of the death to the baby.

- Administrative autopsy
- Judicial autopsy
- Pathological autopsy

2. Handling process of specimen

1) Specimen acquisition from clinics, OPD or operation

2) Specimen transfer and storage

A. instant transfer to pathology department,

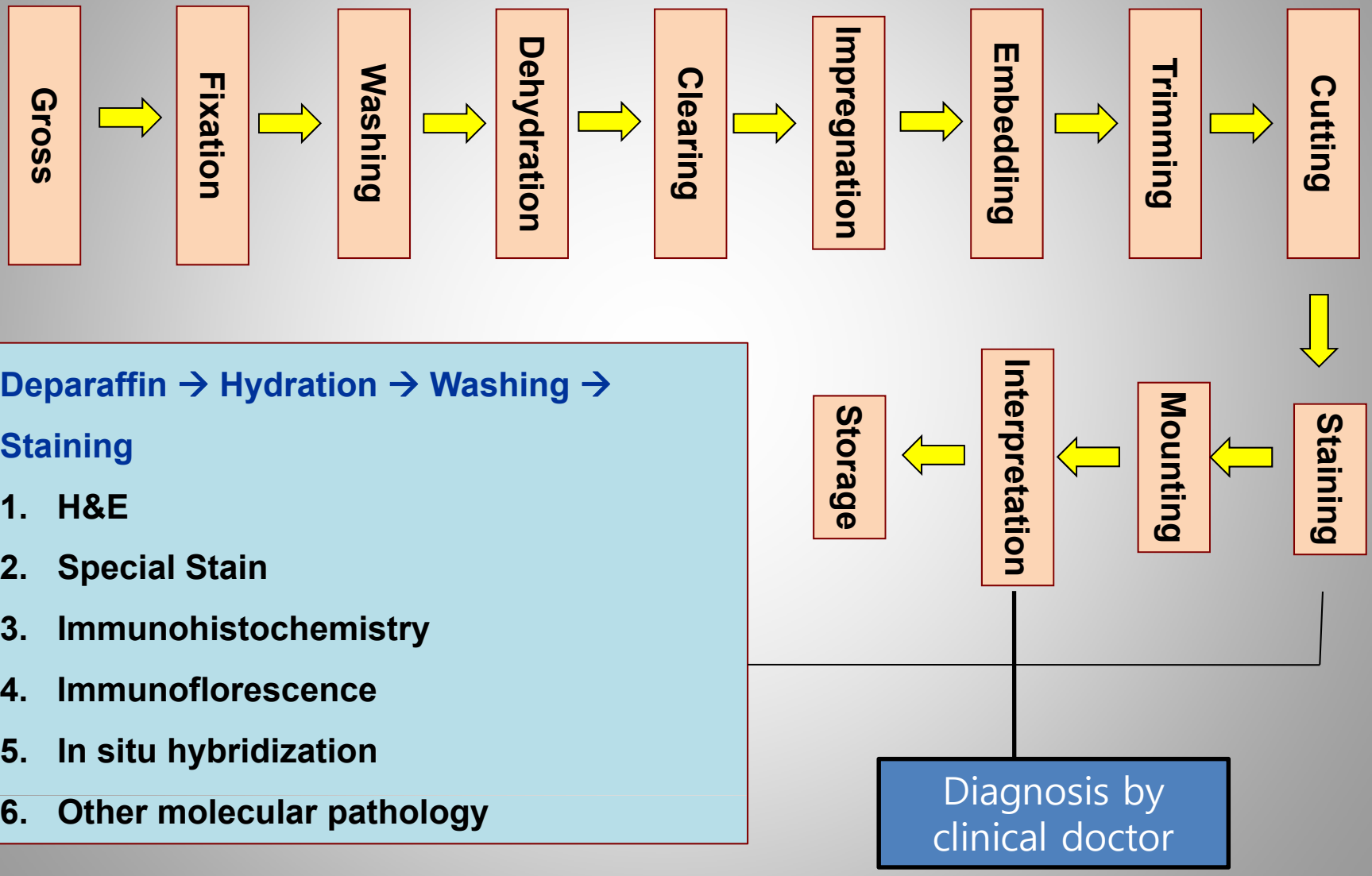
B. fixing in 10% formalin

C. fresh tissue to be stored in a refrigerator at 4°C

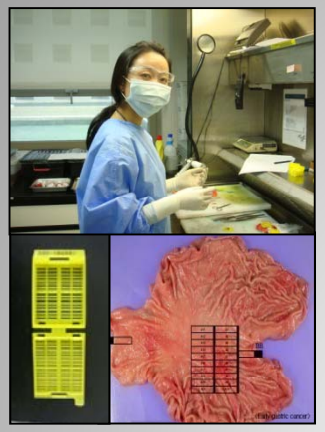
3) Prepare request form of specimen examination : Patients ID no., name, age, sex and name of specimen, operation, diagnosis, history of disease, name of doctor in charge

4) Taking over the specimen after double check on registration numbers and name of patients.

Tissue specimen preparing process



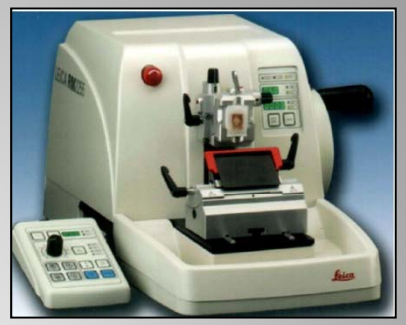
1. Gross (Specimen cutting）



2. Tissue processor

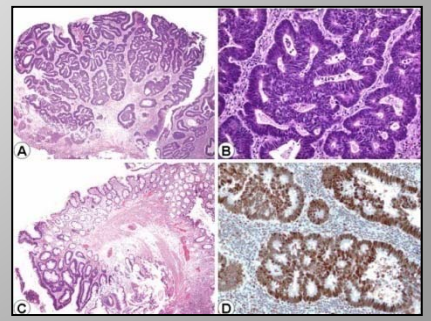
3.Embedding

4. Cutting : Microtome



5. Stain & Mount

6. Interpretation



Frozen ：Report (within 20~30min.)

4. Tissue specimen produce *(pathological inspection process)*

(1) Gross

- Describing opinion on specimen

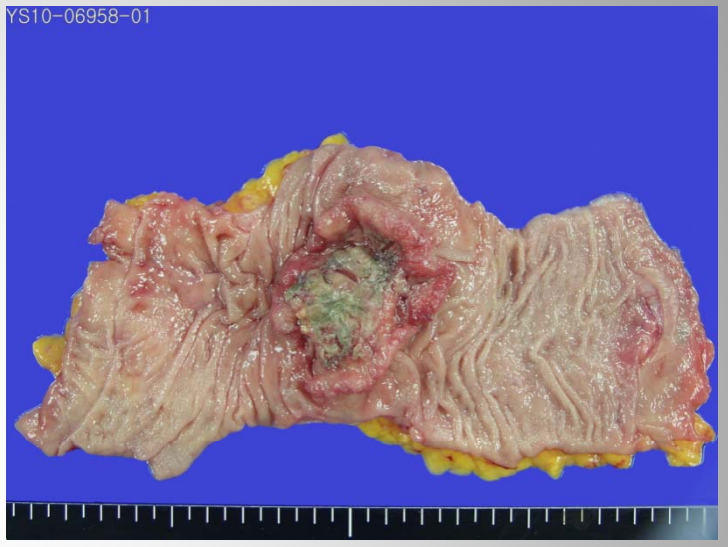
  A. type & structure

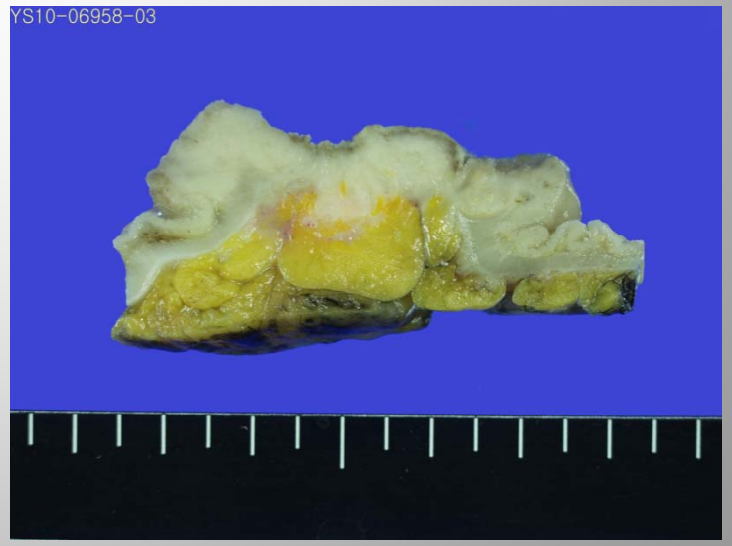
  B. size & weight

  C. shape & color

  D. other differentials

- Photographing & grossing a target tissue (size : 1x2x0.4cm)





(2) Fixation

- Function of fixation
  - Protein solidification(into a semisolid state)
  - Sterilization & protection from rotting, decomposition
  - Maintaining structure of an organism as it is
  - Facilitation or mordanting with biochemical stain
- Requirements of fixing fluid
  - Preserving
  - Hardening
  - Killing
  - Penetrating
  - Mordant

- Kinds of fixing fluid
  - 10% Formalin(4% Formaldehyde)
  - Zenker (pre-staining Dezenker): to fix liver, spleen, fiber, nucleus
  - Helly : to fix tissue with blood
  - Bouin : to fix embryo bud, fetus, testicles
  - Flemming : to fix fat
  - Carnoy : good for glycogen preservation
  - Glutaraldehyde, Osmium tetroxide : fixture for electronic microscope
  - Kaisering : to preserve gross organs permanently for education

✓**Decalcification**

: when target tissue contains bone, calcified focus and calculus in the kidney contains calsium, decalcification process is necessary.

1) Decalcification with acidity

- Nitric acid, HCl, Chromic acid, Formic acid, Trichloroacetic acid

2) Decalcification with electrolysis

3) Decalcification with ion exchange resin

4) Decalcification with radiation

5) others

(3) Washing

Deletion of formalin coloring matter and main ingredient of fixture which are contained in the tissue.

(4) Dehydration

Deletion of moisture contained in the tissue

: as penetration dose does not mix with water, complete dehydration is required

(low density alcohol---> high density (99.9%)alcohol)

(5) Clearing

: Process to impregnate wax into tissues

(use matter which fuses with wax and dehydrator well)

- Xylene
- Ceder wood oil
- Chloroform
- Toluene
- Benzen
- others

(6) Impregnation

Process of impregnating wax into tissues in order to be cut evenly in thickness with a microtome

- Liquid wax should be fully impregnated into tissues until saturated
- Clearing material should be substituted by wax.
- Impregnation should be performed at 2~3°C higher than wax melting point.
- Paraffin

(7) Embedding

Process of forming paraffin block for easy cutting of tissues

- Use ‘Embedding center’
- Paraffin

(8) Trimming

Process of trimming the periphery of paraffin block for easy adapt to microtome.

(9) Cutting

Cut paraffin block with a microtome into a even thick sheets

- Cutting thickness : 4～8㎛ (varies depending on specific purpose)
- Kinds : Sliding microtome, Rotary microtome

(10) Stain

Process of verifying morphologic transformation of tissue structure and revelation of protein through physical, chemical, chemicophysical, immune reaction.

- Hematoxylin & Eosin stain
- special stain
- Immunohistochemistry
- Immunoflorescence
- in situ hybridization & others others

√ ***common process with all stain***

Deparaffin -> Dehydration -> Washing -> Staining

(11) Mounting

Process of sealing with cover glass and mounting matter.

- Requirement of mounting matter (material)

  : transparency, index of refraction, neutral (fluid)

- Purpose

  - prevention of discoloration

  - prevention of decomposition

  - protection from damage to tissues

- Kinds

  - water soluble mounting matter

  - non-water soluble mounting matter

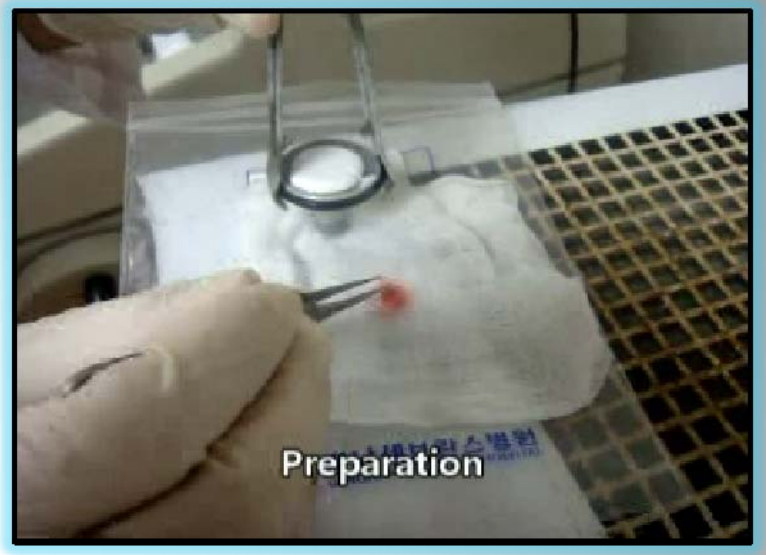
√ Cryo-section

- Purpose

  1) Prompt diagnosis during operation

  2) Attestation of several enzym and lipid

  3) Immunohistochemistry



- Cryo-microtome (-15 ～ -24℃)

  1) CO_2 cryo-microtome : Sartorius type – function of vaporizing heat

  2) Cryo-state: Electro type

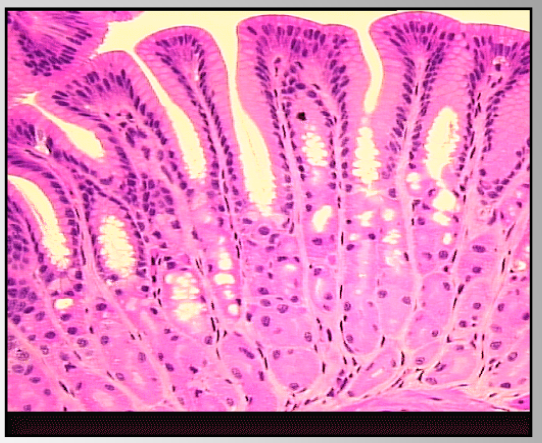
Ⅲ. Staining

1. Hematoxylin-Eosin stain

- Most popular stain method for tissue stain
- verification of tissue components

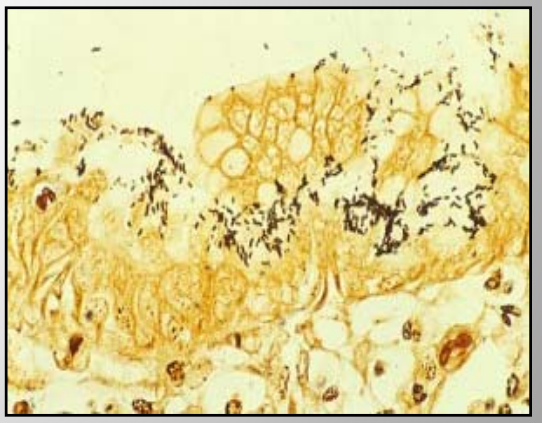
✓ nucleus : blue

✓ fiber, conjoined tissue : orange



2. Special stain

- discrimination of peculiarity in microstructure
- to discriminate peculiar matter
- staining after selection of dye through various immunochemistry



- epidermis, conjoined tissue, muscle, nerve fiber, etc.

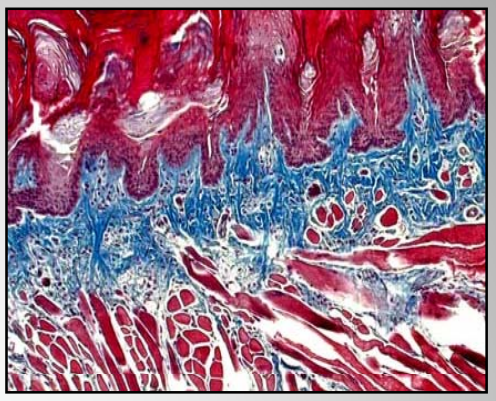
1) conjoined tissue

A. Collagen fiber

Mallory P.T.A.H stain

Masson`s trichrome

Van Gieson`s collagen fiber

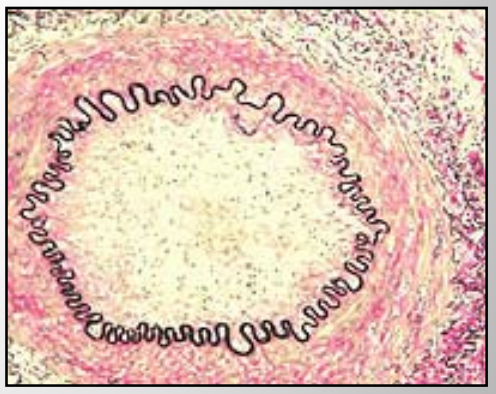


B. Elastic fiber

Verhoeff`s elastic fiber

Orcein`s elastic fiber

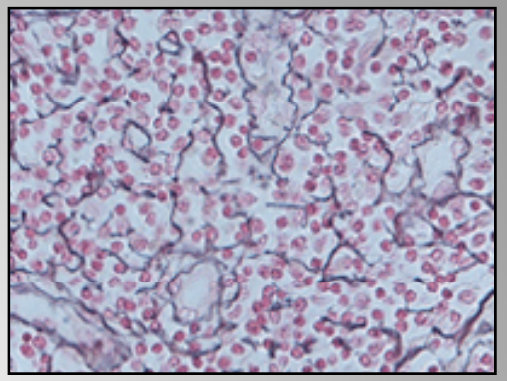
Gomori`s aldehyde fuchsin stain



C. Reticulum fiber

Gomori`s reticulum stain

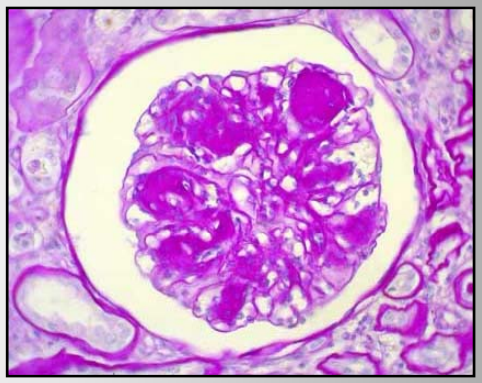
Pap`s Silver reticulum stain



D. Basement membrane

Jones`s PAMS(periodic acid methenamine silver) stain

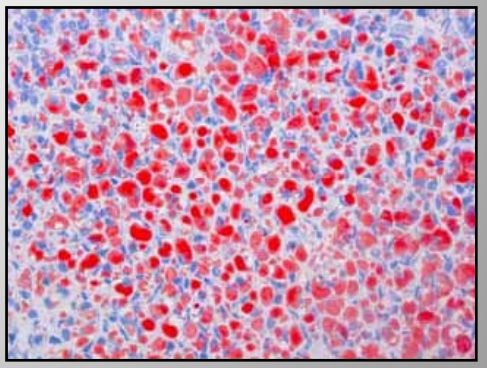
PAS(periodic acid Schiff) stain



2) Lipid

Oil red O stain

Sudan black B stain

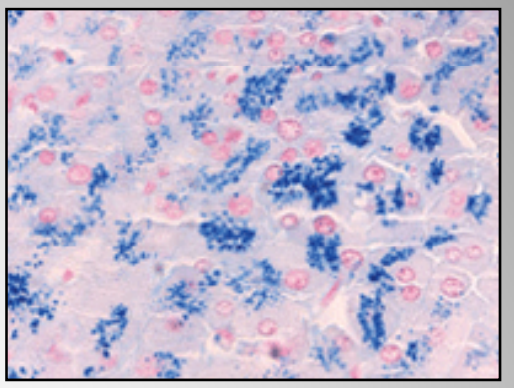


3) Inorganic

Kossa`s calcium stain

Perl`s iron stain

Rhodanine method for copper stain



4) Mucin & Glycogen

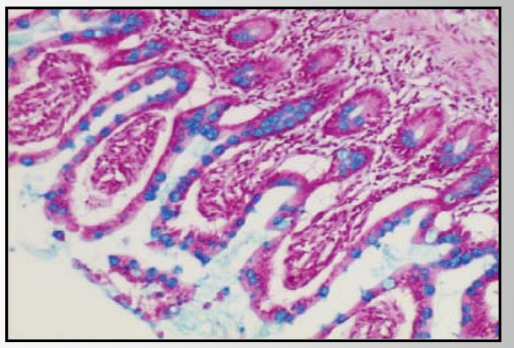
PAS (periodic acid schiff `s) stain

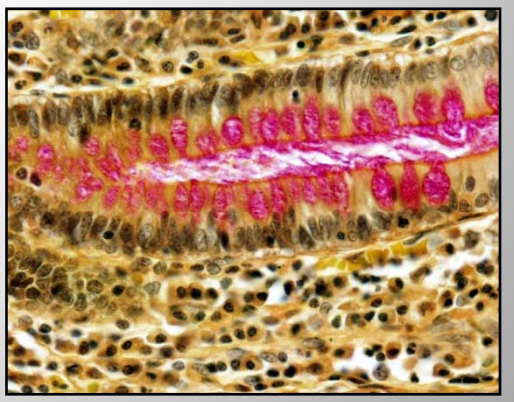
Diastase - PAS Stain

Alcian blue stain

Alcian blue - PAS double stain

Mucicarmine stain





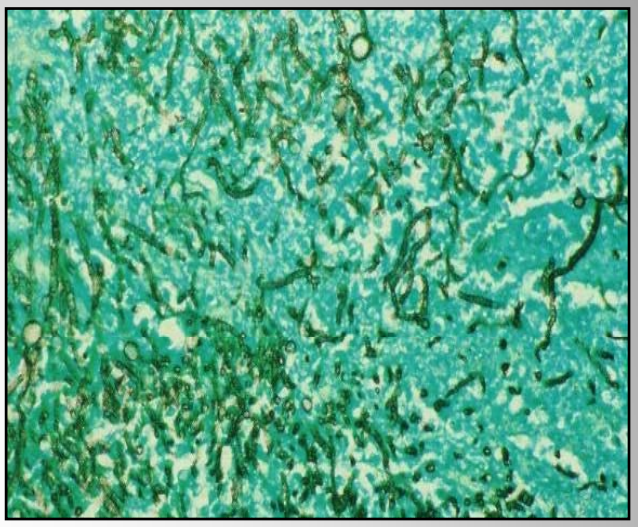
5) Germ, true fungi & cell membrane

Gram stain(Brown brenn, Brown hopps)

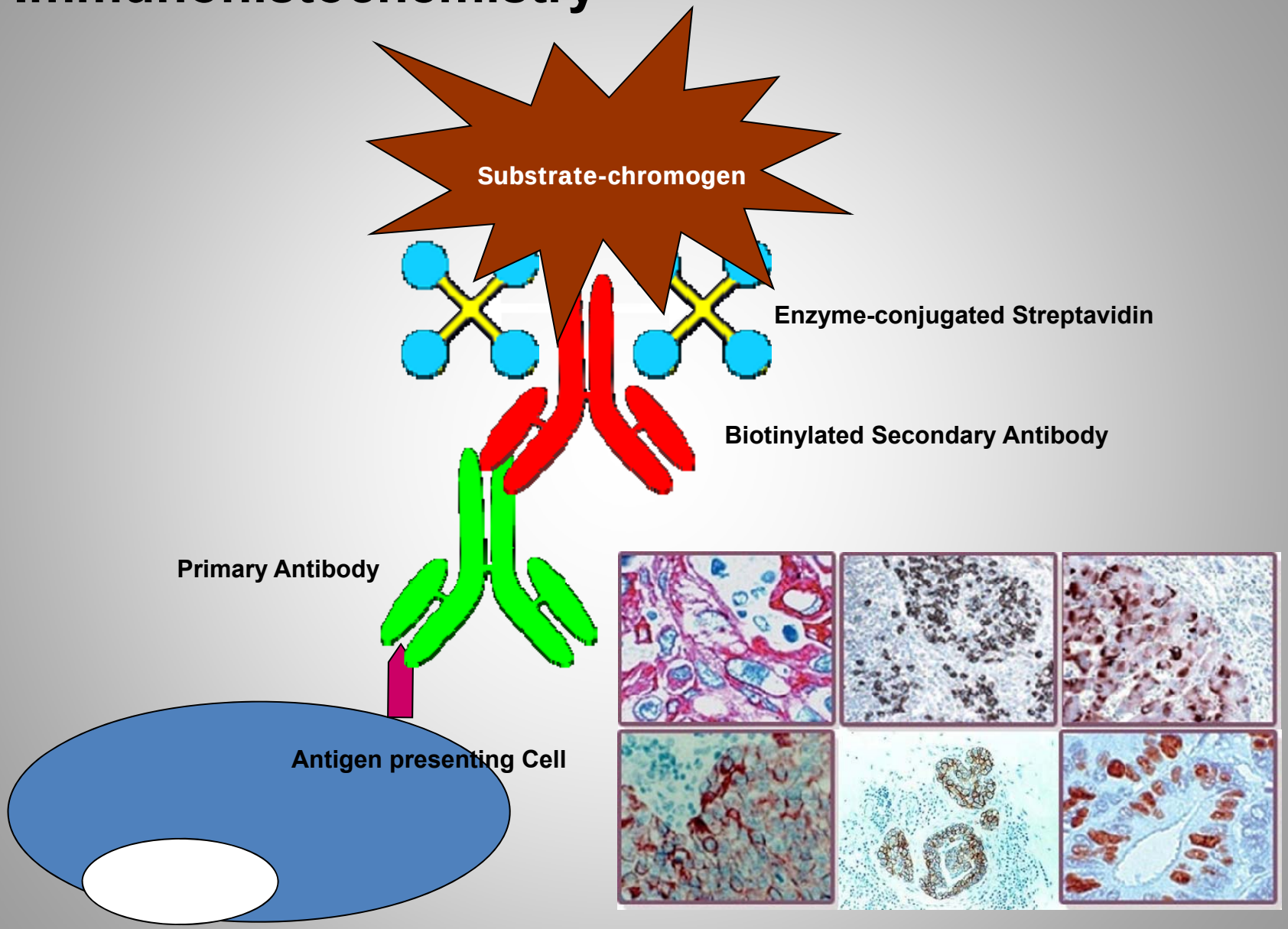
Ziehl-neelsen`s acid fast bacilli(AFB) stain

Grocott`s Methenamine silver stain

Victory Blue stain for HBsAg



3. Immunohistochemistry



4. Molecular biological inspection

- Southern blotting
- Northern blotting
- Western blotting
- PCR(Polymerase chain reaction)
- In Situ Hybridization
- In Situ PCR

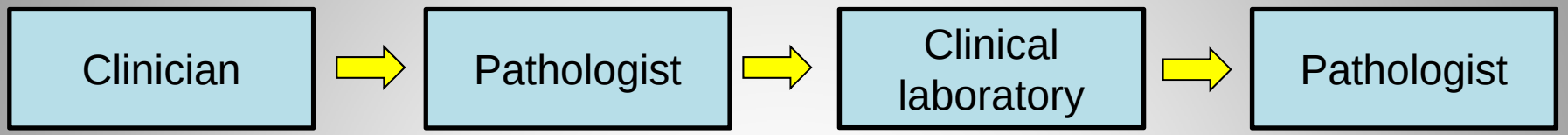
Research with pathological sample tissue

- **Research**
  - Purpose : analyzing disease etiology, prognosis and treatment
  - Statistical purposive decision of research on specific disease group
  - Verification of hypothesis

Ex) 1. Revelation of mutation P53 & PCNA from colon adenocarcinoma and carcinoma

2. Revelation of P63 from lung cancer

Research flow-chart

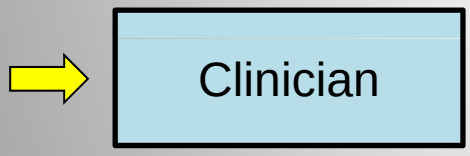


- hypothesis
- Listing patient group
- Decision on research method

- Case review
  : Slide interpretation
- Revising patient group list

- Case review
  : slide & block review
- Perform research
  - H&E stain
  - Special stain
  - IHC
  - ISH
  - others

- interpretation



- Statistics collection & writing thesis

TMA vs General method

Title of thesis : revealation of mutation P53 & PCNA from colon adenocarcinoma and carcinoma

***** patients group : 75 cases with colon adenocarcinoma , 75 cases of carcinoma***