

Introduction to Hospital Pathology and Laboratory Diagnostics

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BCHM4608 (PATHOLOGY SECTION)

Lecture outline

1. Difference between BCHM3606 and BCHM4608
2. Introduction to hospital laboratory investigations
3. Anatomical pathology service in the hospital
 - Histopathology
 - Cytology

Difference between BCHM3606 and Pathology Section of BCHM4608

- BCHM3606 – Focus on theory of different diseases and their biological mechanisms
 - Example: Mr. A (57-year-old) has acute myocardial infarction (AMI)
 - AMI caused by ruptured atherosclerotic plaque in coronary artery of the heart → thrombus forms in coronary artery → blood flow to heart muscle blocked → Heart muscle dies by lack of oxygen → Heart cannot pump blood → Patient goes into shock
 - If you take Mr. A's heart out, you can see the thrombosed coronary artery (by gross examination) and heart muscle cells with coagulative necrosis (by microscopic examination)

Difference between BCHM3606 and Pathology Section of BCHM4608

- BCHM4608 Pathology section – Focus on how to use laboratory investigations to help you make a diagnosis
 - Example 1: Mr. A has sudden chest pain after running, pain radiates to left shoulder
 - You think Mr. A has acute myocardial infarction
 - How to confirm your diagnosis:
 - Electrocardiogram (ECG)
 - Take blood for **troponin I**
 - Why the blood test works: Dead heart muscle cells release troponin I into blood → Blood levels of troponin I becomes high → Troponin I is a **biochemical marker** for acute myocardial infarction

Difference between BCHM3606 and Pathology Section of BCHM4608

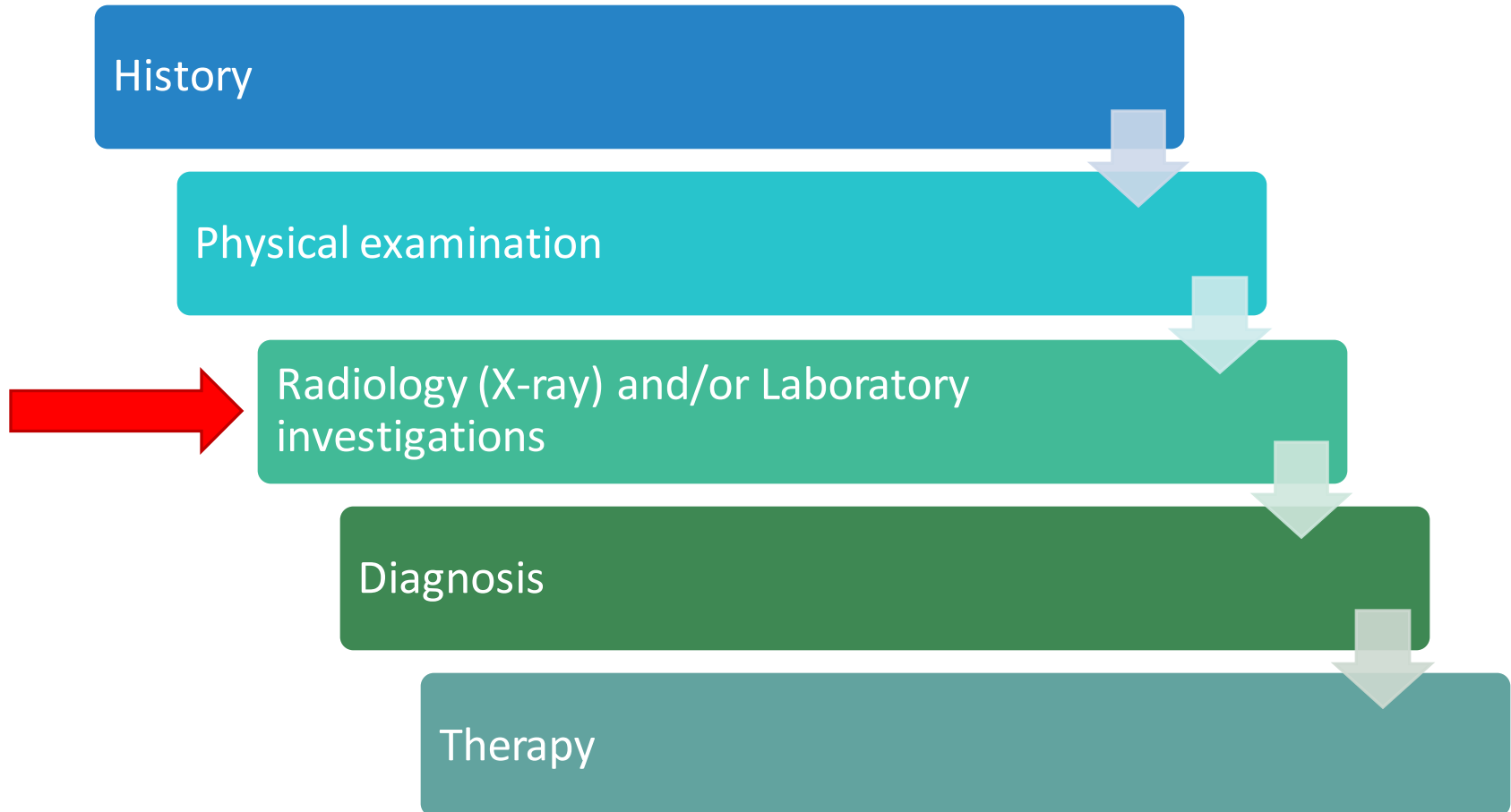
- Example 2:
 - Mrs. B (77-year-old) had fever, cough and shortness of breath for 3 days
 - Auscultation: Crepitations in the right lower zone of the chest
 - You think Mrs. B has pneumonia
 - How to confirm your diagnosis:
 - Chest X-ray
 - Sputum for bacterial culture
 - Nasopharyngeal aspirate for PCR for SARS-Coronavirus-2 (COVID-19), influenza virus and other viruses
 - The bacterial culture and PCR tests are provided by the hospital microbiology lab

Laboratory investigations

- In BCHM4608 (pathology section), you will learn about different laboratory investigations that can help you confirm your diagnosis.
- This is recommended by the Chinese Medical Council of Hong Kong (香港中醫藥管理委員會)

Introduction to laboratory investigations

How to make a diagnosis (in western medicine tradition)



Main hospital laboratories

Anatomical
pathology

Haematology

Clinical
Biochemistry (or
Chemical
Pathology)

Medical
Microbiology

Immunology

Types of hospital laboratories

Laboratory	Main types of investigations
Anatomical Pathology (組織病理 / 解剖病理)	<ul style="list-style-type: none">• Cells from tissue fluids (e.g. sputum, urine, pleural fluids, etc.) → To look for cancer cells• Small pieces of tissue removed by endoscopy or surgery (biopsy) → To look for cause of the disease or any cancer cell• Organs removed by surgical operation• Typing, grading and staging of cancer• Autopsy service
Haematology (血液病理)	<ul style="list-style-type: none">• Counting of blood cells in peripheral blood• Blood clotting profile• Bone marrow examination• Blood group typing for blood transfusion• Maintenance of the hospital blood bank for blood transfusion

Types of hospital laboratories

Laboratory	Main types of investigations
Clinical Biochemistry (生物化學) (also called “Chemical Pathology 化學病理”)	<ul style="list-style-type: none">• Electrolytes in blood (e.g. sodium, potassium, calcium, etc.)• Arterial blood gases (oxygen level, bicarbonate, pH)• Cellular enzymes and other chemicals in blood (e.g. Liver function tests, acute myo)• Hormone levels in blood• Drug levels in blood and toxicology• Genetic testing for inherited diseases

Types of hospital laboratories

Laboratory	Main types of investigations
Medical Microbiology (微生物學)	<ul style="list-style-type: none">• Bacterial culture from patient specimens (e.g. blood, sputum, urine, faeces, other body fluids, tissue, etc.)• Antibiotic susceptibility testing for bacteria• Testing for tuberculosis and other acid-fast bacilli• Viral studies by PCR and/or culture• Fungal studies• Serology (blood antibodies level) for infections

Types of hospital laboratories

Laboratory	Main types of investigations
Immunology (免疫學)	<ul style="list-style-type: none">• Auto-antibodies in blood for autoimmune diseases (e.g. systemic lupus erythematosus, rheumatoid arthritis, connective tissue diseases, etc.)• Antibodies in blood for allergies• Blood immunoglobulin profile for blood cancers• Genetic (HLA) typing for organ transplant

How to order lab investigations

1. You have a clinical question that needs to be answered by blood test, urine test, or other lab tests
2. Collect a specimen (標本) from the patient
 - Blood
 - Sputum
 - Urine
 - Faeces
 - Cells and tissue (from surgery), etc.
3. Put in appropriate container
 - Different blood tests require different types of blood collection tubes, please check with the lab
 - Sputum, urine, etc. can be put in sterile contain supplied by the lab
4. Label the container/blood collection tube with patient's information
 - Patient's name, sex, age, ID number
 - If not blood: Also write down the site of collection on the label

How to order lab investigations

4. Fill in request form for the lab
 - Patient particulars, such as name, sex, age, date of birth, ID number
 - Your own contact information (when ordered outside a hospital)
 - Information on the specimen
 - Specimen collection date
 - Type of specimen (blood, urine, faeces, etc.)
 - Clinical history of the patient
 - The type of test that you want to order
5. Send the specimen to the lab (usually via a porter from the lab)

Cytology specimens in Queen Mary Hospital

- AP201709 -

Request form example

Microbiology specimens in Queen Mary Hospital

DEPARTMENT OF MICROBIOLOGY • QUEEN MARY HOSPITAL MICROBIOLOGY INVESTIGATION FORM

Collection date: _____ Time: _____
(dd/mm/yyyy)

2nd Label

Biohazards: Unknown / Known (please specify) _____

Affix patient's gum label or fill in the details:

HKID number: _____ AE HN: _____

Surname: _____ First name: _____

Sex / Age: _____ Ward / Bed: _____

Clinician in-charge / Unit: _____ Outpatient ☐
Private ☐

Requesting clinician (name in block letters): _____

QMH staff no.: _____

SPECIMEN TYPE

(For viral investigations, place specimen in virus transport medium)

☐ Abscess/Pus (specify site) CSF: ☐ LP / EVD
☐ VP / VA shunt

Aspirate Enteric: ☐ Stool
☐ Rectal swab

(for viral investigations only)

☐ Gastric ☐ Ascitic
☐ Others ☐ Pericardial

☐ Biopsy (specify site) ☐ Peritoneal
☐ Bone (specify site) ☐ Peritoneal dialysis

☐ Bile: (specify site) ☐ Pleural
☐ Others ☐ Joint

Blood: ☐ Culture
☐ Clotted ☐ Reverse Osmosis (RO) water
☐ EDTA ☐ Pre-dialysate

Intravascular catheter ☐ HDF Infusate (100 mL)
☐ Broviac/Hickman ☐ AER (100mL)
☐ Others

Date received: _____

Lab no. _____

Clinical details: _____

Receiving antimicrobials?
Please specify. _____

Respiratory ☐ Sputum ☐ Day 1 / ☐ Day 2
☐ BA ☐ BAL
☐ ETA ☐ TA

Urine ☐ Bag urine
☐ CSU (indwelling)
☐ CSU (cath once)
☐ EMU (AFB only)
☐ MSU
☐ Nephrostomy
☐ -date of tube insertion:
☐ Ureteric
☐ -date of cytology: _____

Tissue ☐ (specify site) _____

Swabs Ear ☐ Rt ☐ Lt ☐ both in 1
Eye ☐ Rt ☐ Lt ☐ both in 1
☐ Nasal
☐ Nasopharyngeal
☐ Perinasal
☐ Oral
☐ Throat
☐ Endocervical
☐ High vaginal
☐ Urethral
☐ Peritoneal
☐ Placental
☐ Umbilical
☐ Ulcer
☐ Superficial wound
☐ Deep wound
☐ Skin
☐ Vesicle

Others (please specify) _____

BACTERIOLOGY, MYCOLOGY, AND PARASITOLOGY INVESTIGATIONS

Smear ☐ AFB

☐ Bacteria
☐ Fungi

Parasites ☐ Amoebae (fresh stool)
☐ Ova and cysts
☐ Trichomonas
☐ Scabies
☐ Pneumocystis (BAL)
☐ Others (please specify) _____

Culture ☐ Aerobic bacteria
☐ Anaerobic bacteria
☐ Acid-fast bacilli
☐ Fungi
☐ Bordetella pertussis
(pernasal/nasopharyngeal swab only)
☐ Legionella spp.
☐ Neisseria gonorrhoeae

☐ Clostridium difficile culture
☐ Clostridium difficile cytotoxin
Urine ☐ Routine microscopy
☐ Chyle

Others (please specify) _____

VIROLOGY AND MOLECULAR TESTING

Antigen detection ☐ Respiratory viruses
☐ HSV
☐ VZV
☐ CMV pp65 antigenaemia
- Fresh EDTA blood arrived before 1200
- No service on Sat, Sun and PH

Rapid viral culture (V05) ☐ Adenovirus
☐ CMV
☐ HSV
☐ VZV

Others (please specify) _____

Molecular ☐ BK virus
☐ HSV
☐ CMV
☐ EBV (EDTA blood-plasma)
☐ Enterovirus
☐ VZV
☐ Mycoplasma pneumoniae
☐ M. tuberculosis (TB-PCR)

SEROLOGY AND THERAPEUTIC DRUG MONITORING (use clotted blood)

Bacterial ☐ Anti-streptolysin O
☐ Bartonellosis
☐ Brucellosis
☐ Leptospirosis (IgM)
☐ Melioidosis
☐ Syphilis
☐ Tetanus
☐ Typhoid

Fungal ☐ Aspergillus antigen
☐ Aspergillus fumigatus IgG
☐ Cryptococcus antigen
☐ Penicillium marneffei antibody

Viral ☐ Needlestick injury
☐ source ☐ victim
☐ Hepatitis markers
(please specify)

☐ EBV (NPC screening)
☐ EBV (Acute screening)
☐ HIV Serology
☐ Parvovirus B19 IgM
☐ CMV IgM

Immune Status
☐ CMV
☐ EBV
☐ VZV

Parasitic ☐ Amoebiasis
☐ Cysticercosis
☐ Toxoplasmosis
☐ Trichinellosis

☐ Vancomycin trough level

Please specify: _____
Serum creatinine level: _____
Dosage and frequency: _____

Others (please specify) _____

For enquires, please call the laboratory at 2255 2378 (office) or 2255 2387 (laboratory)
**For urgent tests, please notify the laboratory at 2255 2387 before sending specimens

Fax: 2872 4555

Blood Collection Tubes



Machines in the haematology laboratory



Examples of the types of specimens and which lab tests you can order

Specimen	Lab test	Which lab
Blood	<ul style="list-style-type: none">• Complete blood count• Clotting profile (PT, APTT, INR)	Haematology
	<ul style="list-style-type: none">• Electrolytes and renal function test• Liver function test• Blood glucose level• Blood cholesterol and lipid profile• Thyroid hormone levels	Clinical Biochemistry
	<ul style="list-style-type: none">• Auto-immune disease markers	Immunology
	<ul style="list-style-type: none">• Antibody serology for infections	Microbiology
	<ul style="list-style-type: none">• Bacteria culture	Microbiology
Urine	<ul style="list-style-type: none">• Urine pH, protein, white blood cells, red blood cells, nitrite	Microbiology
	<ul style="list-style-type: none">• Look for urinary bladder cancer cells (urine cytology)	Anatomical Pathology

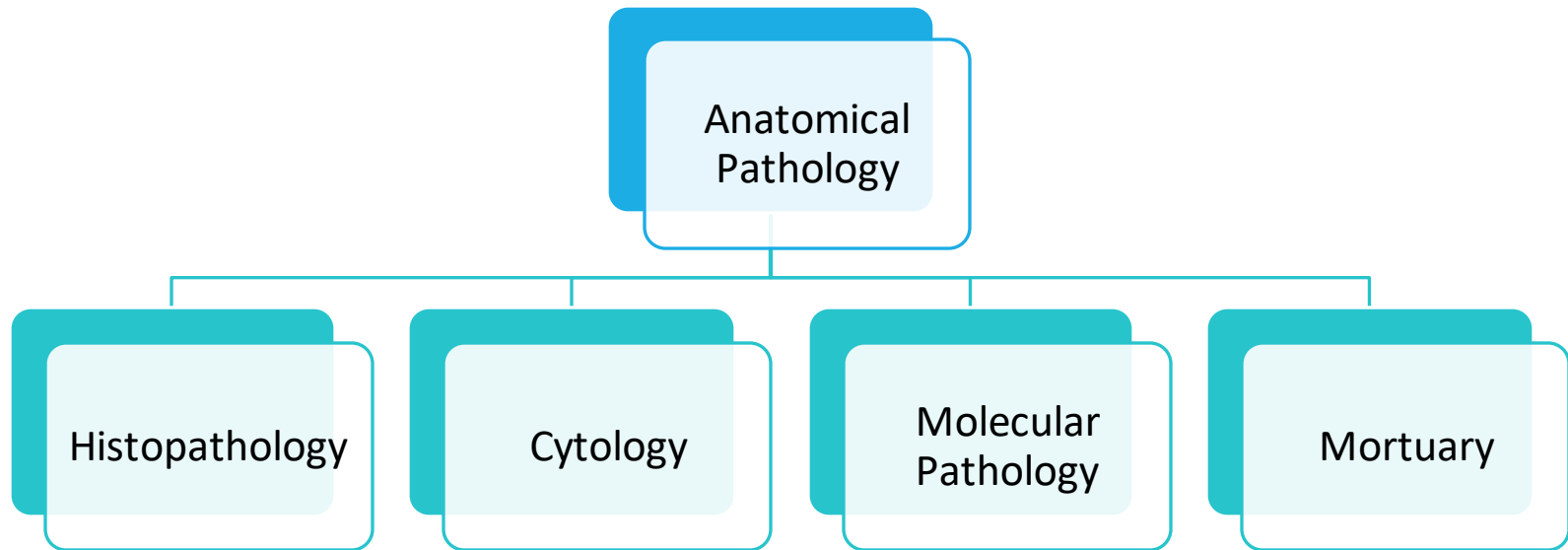
Anatomical Pathology Service in the Hospital

Anatomical Pathology

- Branch of pathology lab that deals with cells, tissue and organs
- We make diagnosis by looking at tissue and cells with microscope



Branches of Anatomical Pathology labs



Branches of Anatomical Pathology labs

Branch	Main duties
Histopathology laboratory	<ul style="list-style-type: none">• Deals with tissue from biopsies and organs from surgical resections
Cytology laboratory	<ul style="list-style-type: none">• Deals with cells from body fluids (urine, sputum, pleural fluids,• Cells from fine needle aspiration
Molecular pathology laboratory	<ul style="list-style-type: none">• DNA testing from cancer cells• Germline DNA testing from blood samples (mainly for hereditary cancer syndromes)
Mortuary	<ul style="list-style-type: none">• Storage of bodies until claimed by family for funeral• Autopsy service

Histopathology Laboratory

Types of specimens we receive

- Small biopsies:
 - Core biopsies of superficial lesions (e.g. breast tumours, lymph nodes)
 - X-ray / CT scan guided biopsies (e.g. lung tumours, intra-abdominal tumours)
 - Bronchoscopy (e.g. lung cancer biopsies)
 - Upper gastrointestinal endoscopy (oesophagus, stomach, duodenum, pancreas)
 - Colonoscopy (colon polyps, colon tumours, inflammatory bowel disease)
 - Endometrium, cervix and vaginal biopsies
 - Prostate biopsies

Histopathology Laboratory

- Large organ resections for cancer
 - Confirmation of tumour type, tumour grade and tumour stage
 - Confirmation of surgical margin status
- Large organ resections for non-cancer diseases
 - Example: Intestinal obstruction → Gangrenous intestine → cut away by surgeons
 - Large uterine fibroids with pressure symptoms or menorrhagia → uterus removed for symptom relief

Histopathology Lab techniques

1. Fixation of specimens and tissue processing
2. Haematoxylin and eosin (H&E) staining
3. Special biochemical stains
4. Immunohistochemistry
5. Molecular pathology tests (send to molecular pathology lab)

Tissue processing

Tumour tissue is fixed in neutral-buffered formalin



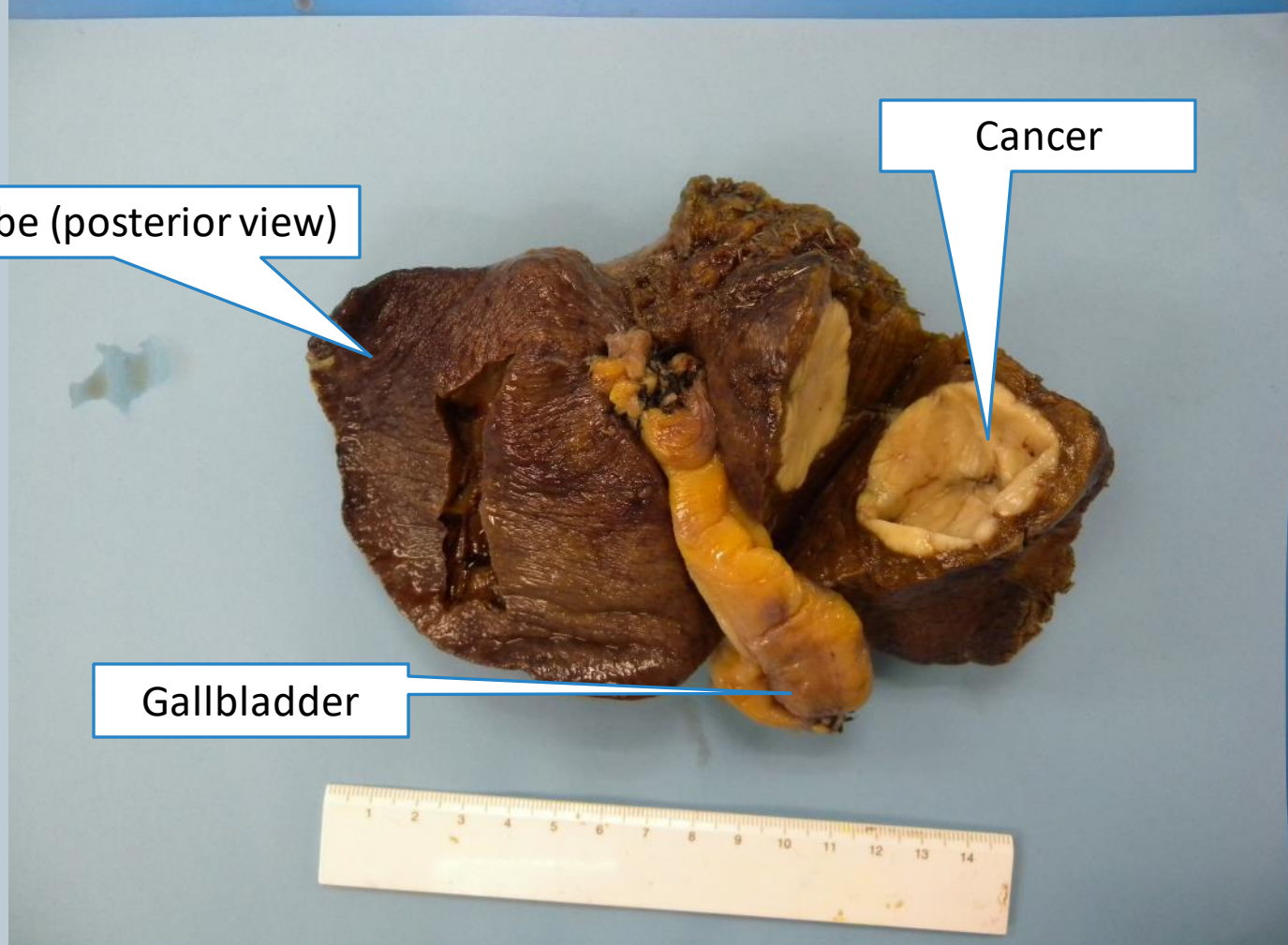
Embedded in paraffin block



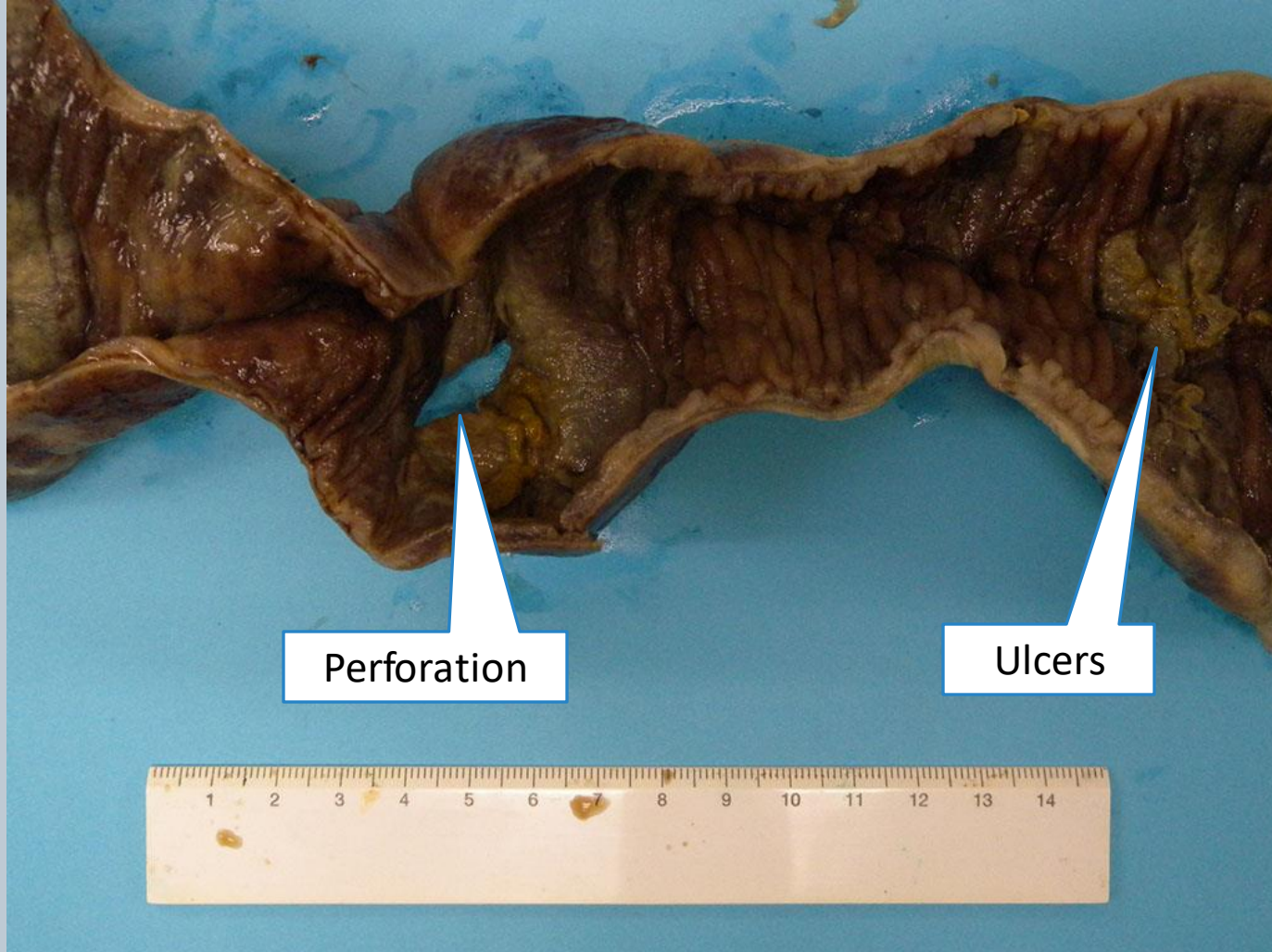
4 μm sections cut from the paraffin block, mounted on a glass slide



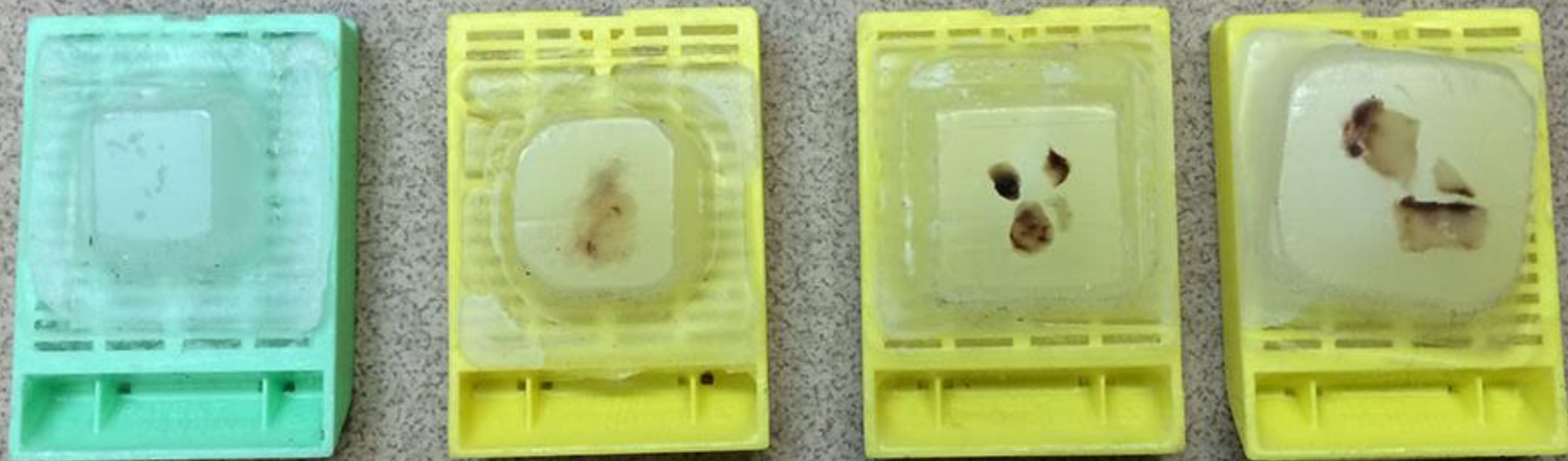
Stained with haematoxylin and eosin



Example of resection specimen:
Liver cancer resection



Example of resection specimen:
Small intestine perforation



Paraffin blocks



Making microscope slides



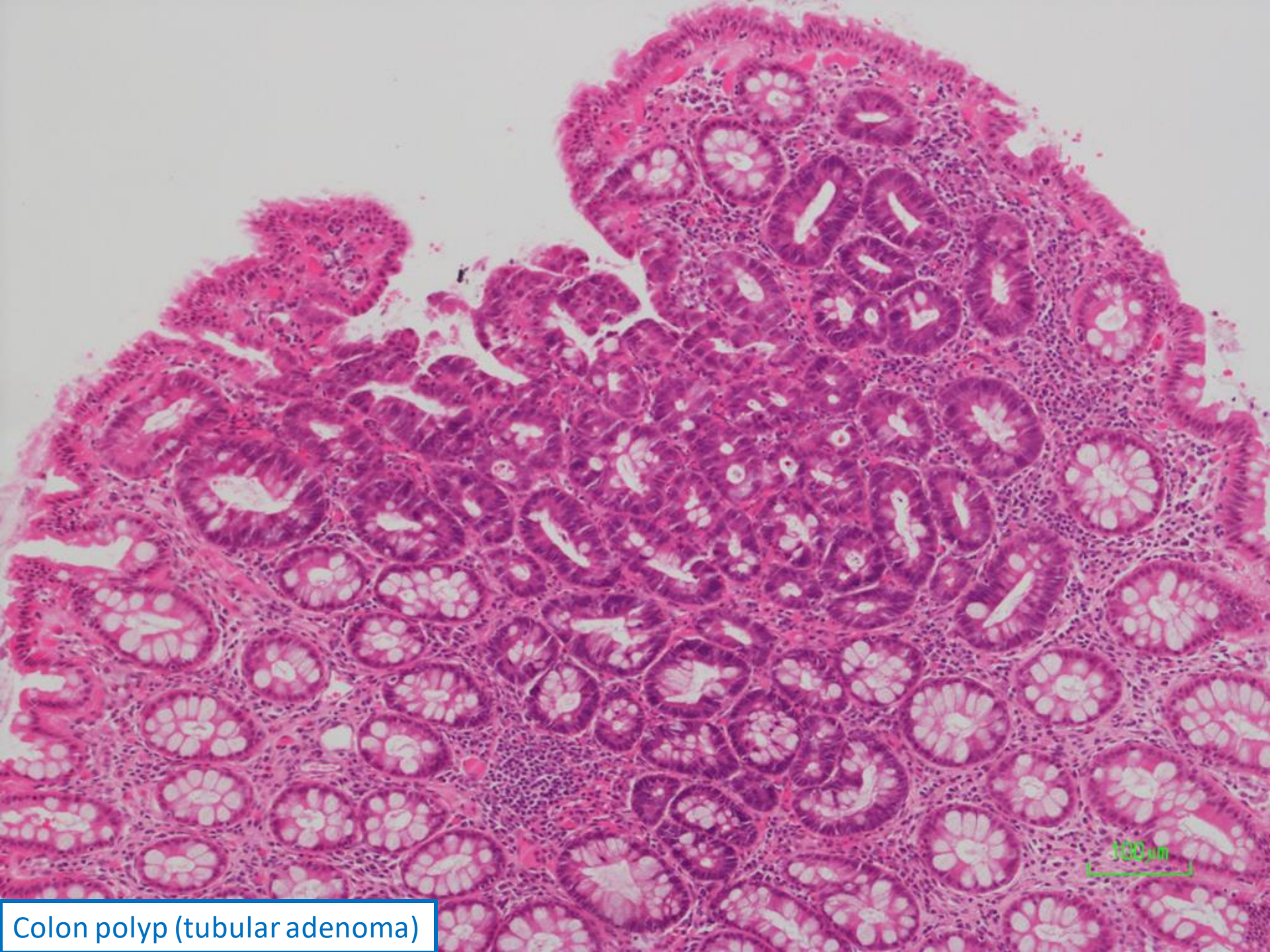
Microscope glass slides



Microscope room in Anatomical Pathology lab

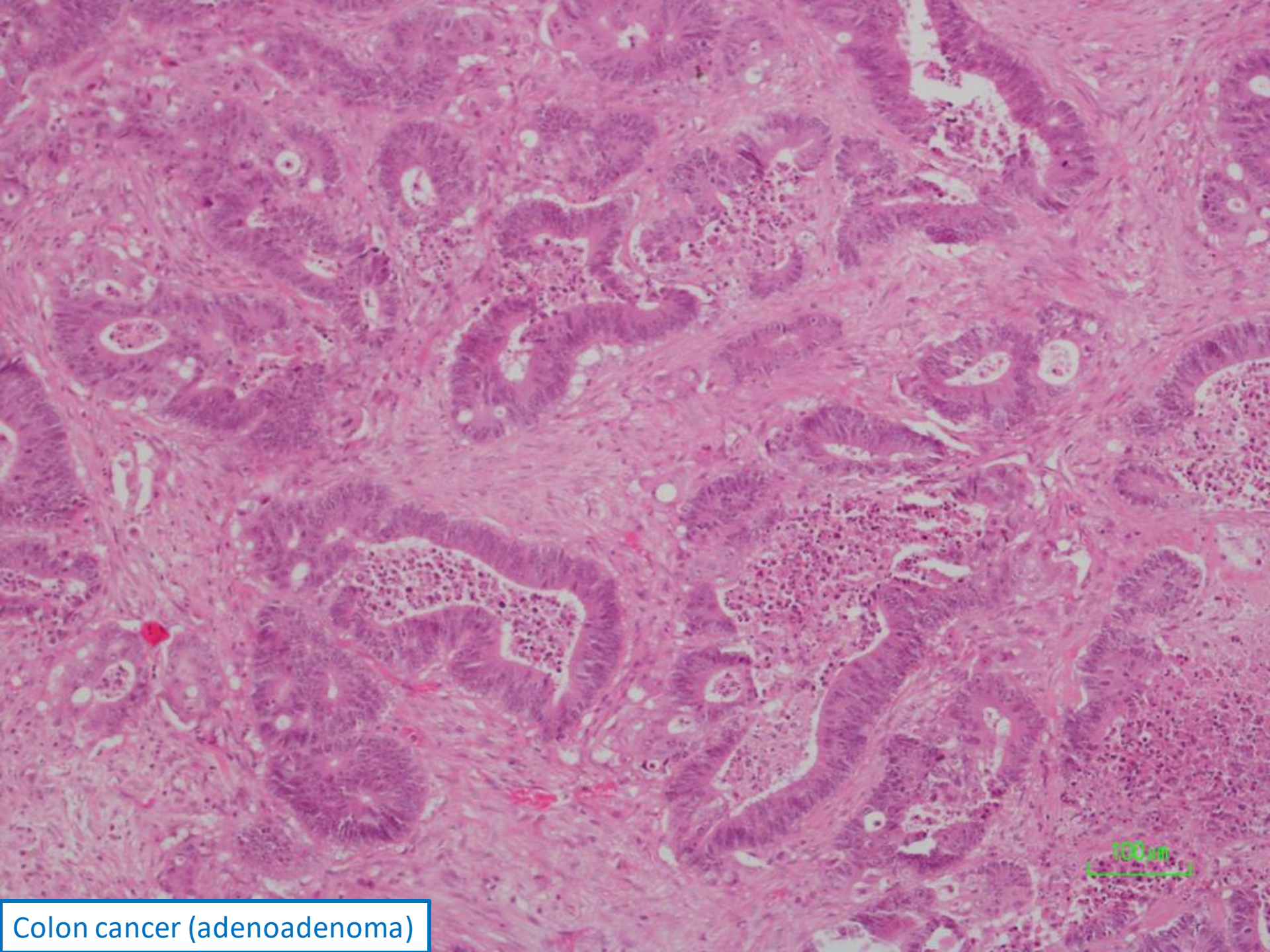
H&E staining

- The basic “purple and pink” stain for microscope slides
- Basic evaluation of tissue architecture and cell morphology
- For many cases, H&E staining already enough for tissue diagnosis



100µm

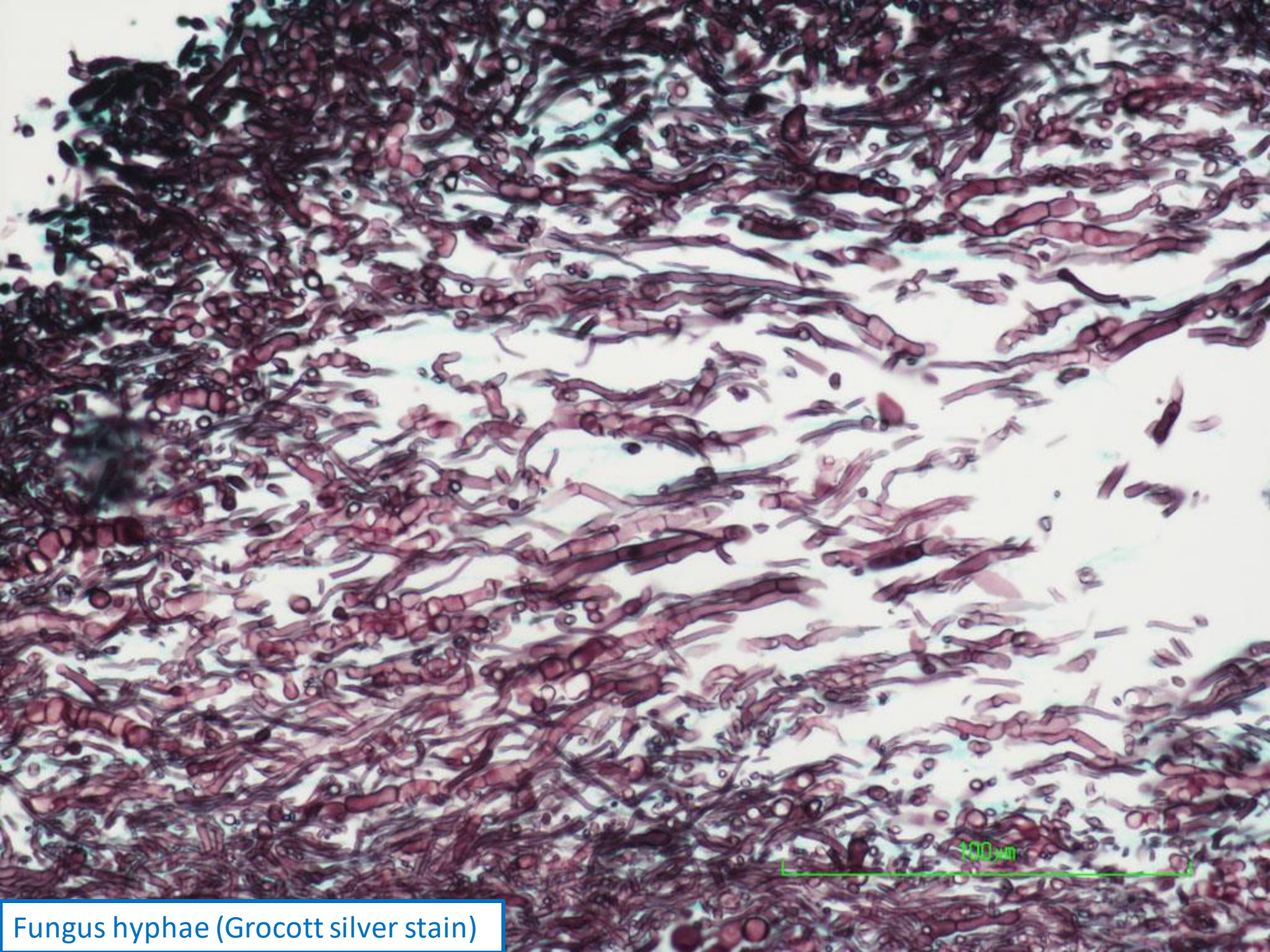
Colon polyp (tubular adenoma)



Colon cancer (adenocarcinoma)

Special biochemical stains

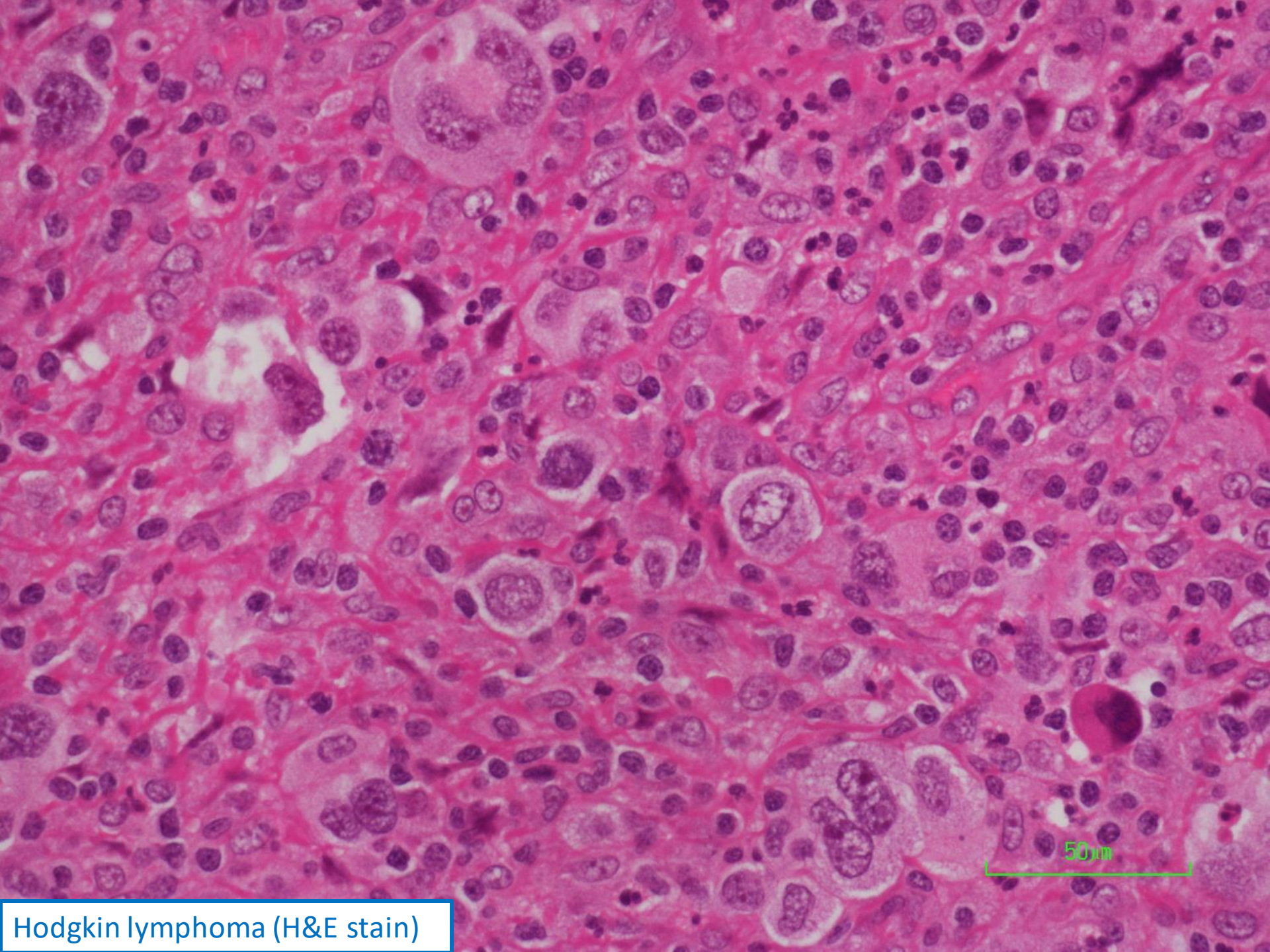
- Used to visualize special structures in the tissue
- Special chemical structures:
 - Collagen
 - Reticulin fibres
 - Mucin
- Mineral deposits
 - Iron (haemochromatosis)
 - Calcium
 - Copper (Wilson's disease of liver)
- Micro-organisms
 - Bacteria
 - Fungus



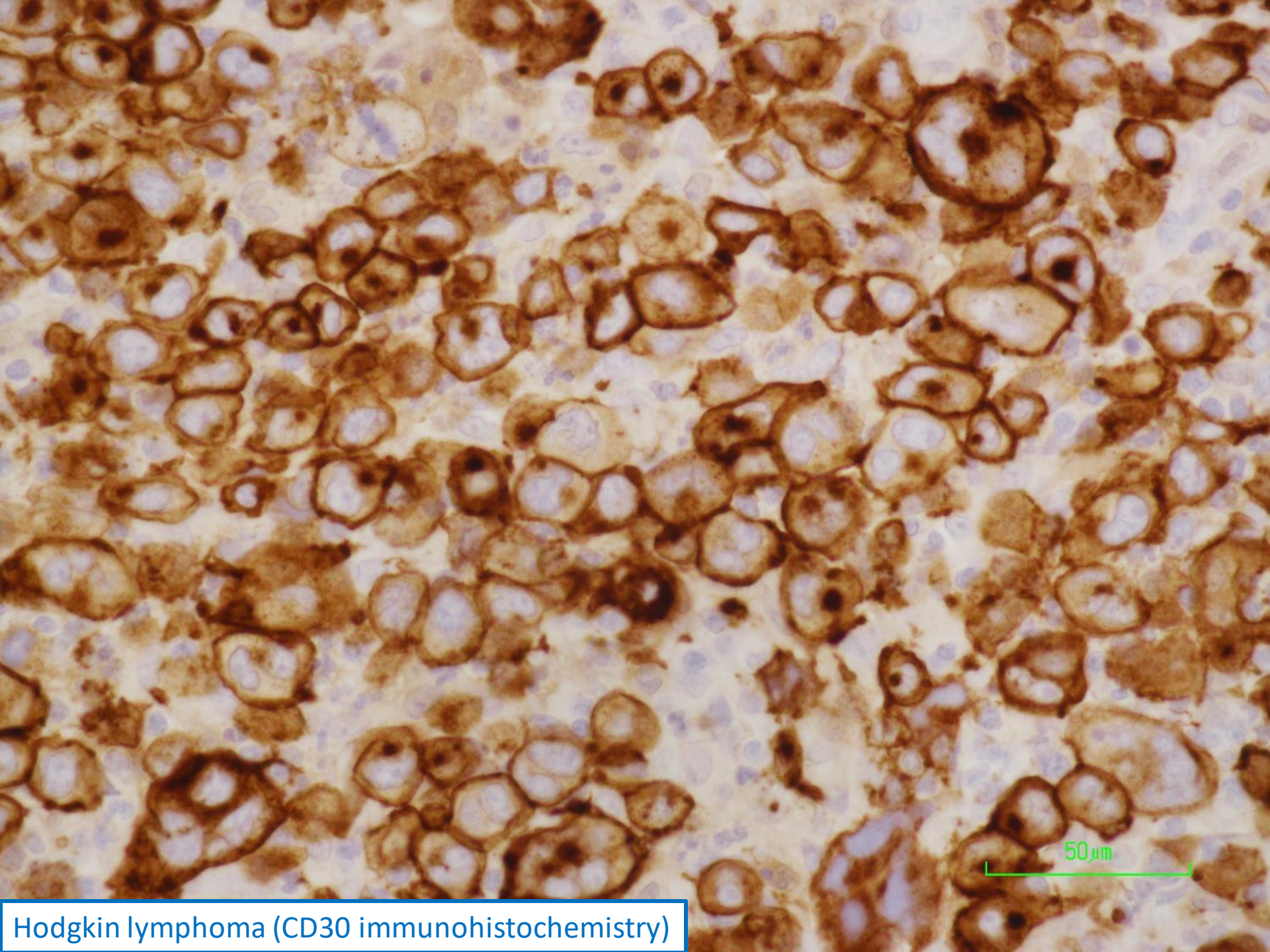
Fungus hyphae (Grocott silver stain)

Immunohistochemistry

- Used to check protein expression in cells
- Uses:
 - Typing of tumour
 - Investigate for possible origin of tumour in metastatic cancer
 - Check possible response to targeted therapy (標靶治療)
 - Look for viral infections in cells (e.g. cytomegalovirus)



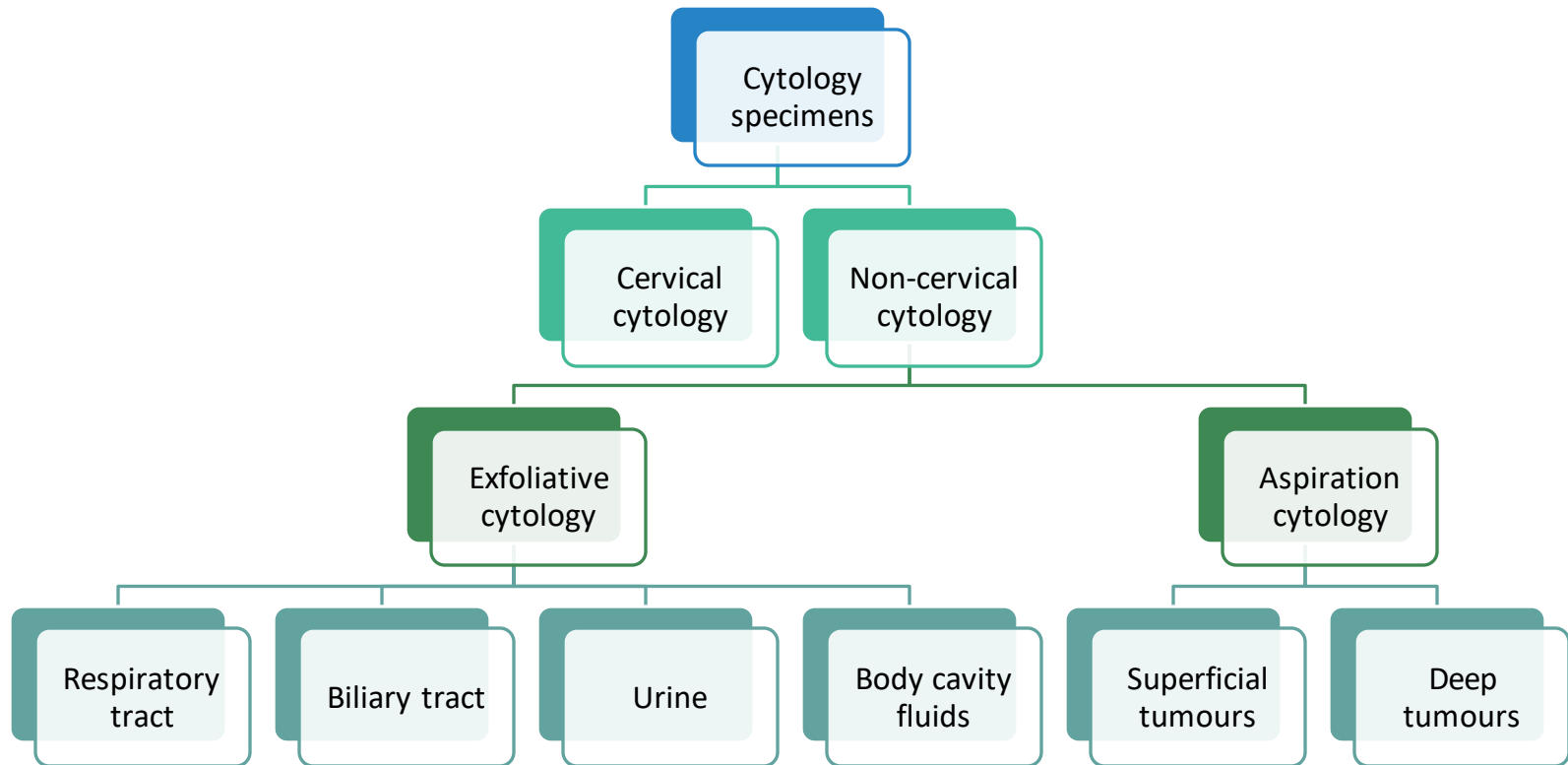
Hodgkin lymphoma (H&E stain)



50 μ m

Hodgkin lymphoma (CD30 immunohistochemistry)

Cytology Laboratory



Cervical cytology

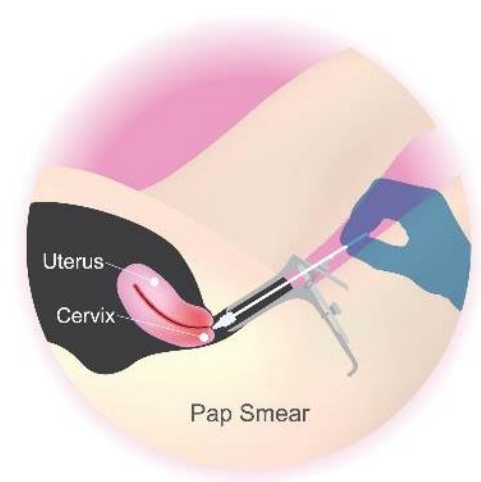
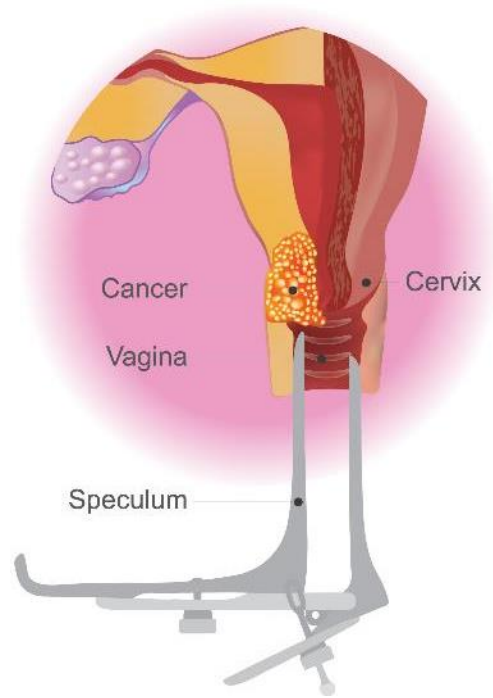
- Screening programme for cervical cancer
- Cervical smear recommended every three years after 25 years old or after sexual activity (whichever later)
- Cervical cells collected by plastic brush → smeared on glass slide or suspended in collection medium (special liquid to preserve the cells)
- Cases without abnormal cells – Reported by cytotechnologists
- Cases with abnormal cells – Reported by pathologists

Main use of cervical cytology: To look for cervical cancer cells

Details on the Hong Kong Cervical Screening programme:

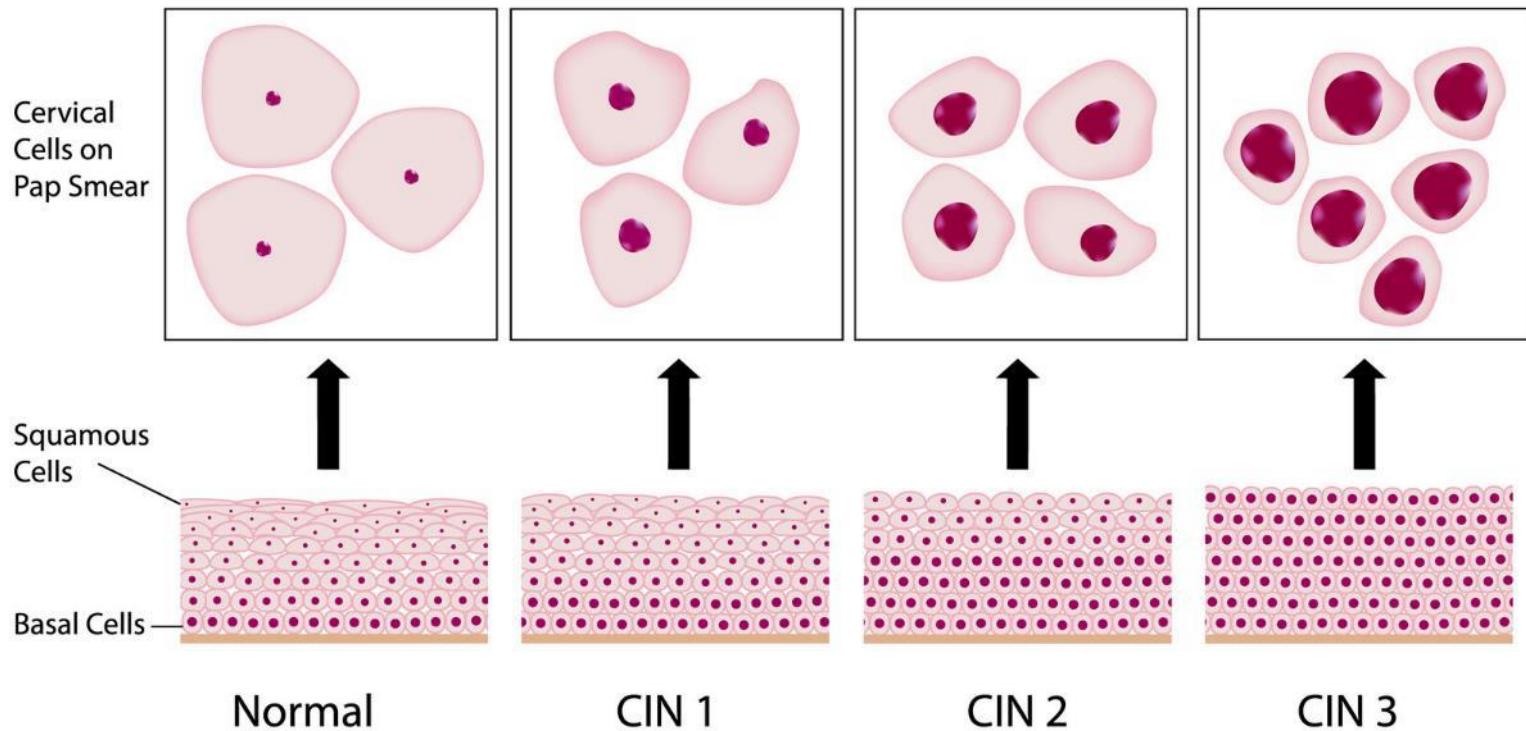
<https://www.cervicalscreening.gov.hk/en/recommendations.html>

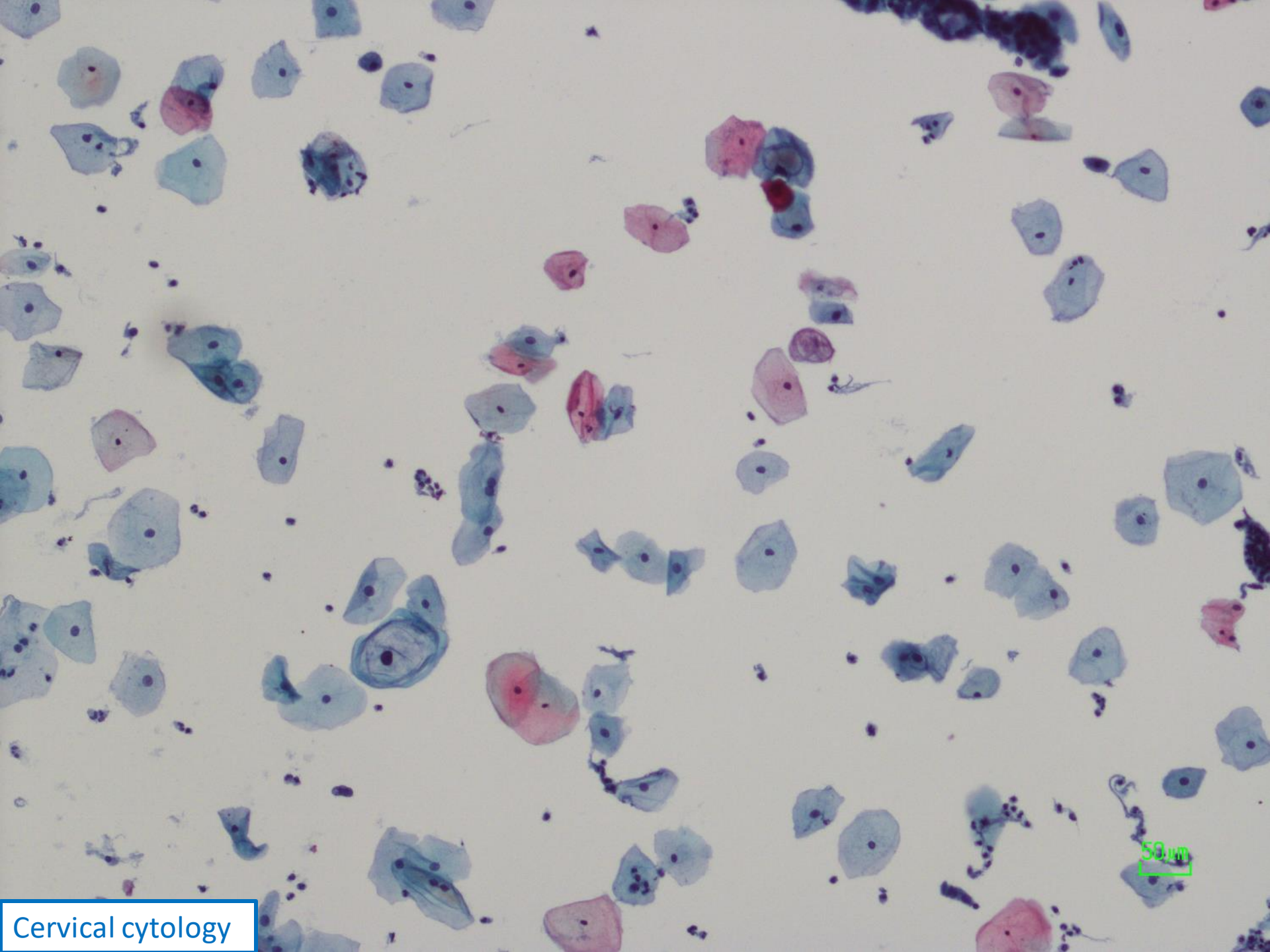
Cervical smear collection



Cervical cytology

Cervical Intraepithelial Neoplasia (CIN)





50µm

Cervical cytology

Cervical cytology results

Cytology result (simplified)	Meaning	Recommended action
Negative	Cells normal	Repeat cervical cytology according to normal schedule
Atypical squamous cells of undetermined significance	Some abnormal cells present, significance uncertain	Repeat cervical cytology in 3 months
Low grade squamous intraepithelial lesion (CIN I)	Human papillomavirus (HPV) infected cells present	Refer to gynaecologist for assessment
High grade squamous intraepithelial lesion (CIN II to III)	Cancer precursor cells present	Refer to gynaecologist for assessment
Squamous cell carcinoma	Cancer cells present	Refer to gynaecologist for assessment
Atypical glandular cells	Some abnormal cells present, from endocervix or endometrium	Refer to gynaecologist for assessment
Adenocarcinoma	Cancer cells present	Refer to gynaecologist for assessment

No need to memorize this table for examination

Non-cervical cytology

- Two main types: Exfoliative cytology and aspiration cytology
- Exfoliative cytology:
 - Examination of cells dropped off or brushed off from luminal surface
 - Main types:
 - Respiratory tract
 - Biliary tract
 - Urinary tract
 - Body cavity fluids
- Aspiration cytology:
 - Examination of cells aspirated by needle
 - Two main types:
 - Superficial tumours and lesions – Aspirated by doctor using palpation or ultrasound guidance
 - Deep tumours (intra-thoracic or intra-abdominal) – Aspirated under CT guidance

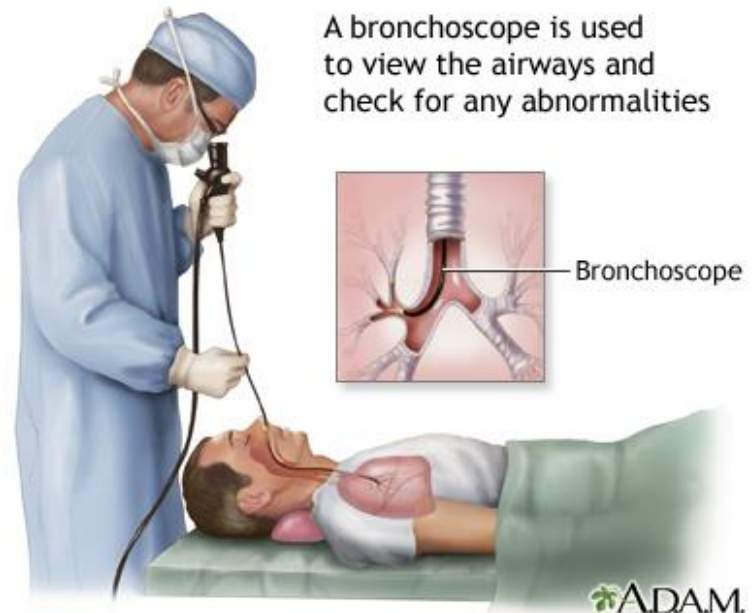
Respiratory cytology - types

Main types	Features
Sputum cytology	<ul style="list-style-type: none">• Cells coughed up by patients• Easy to collect• Sensitivity limited
Bronchial brushing	<ul style="list-style-type: none">• Cells collected by brushing the bronchial mucosa during endoscopy• Difficult to collect• More sensitive than sputum cytology
Bronchio-alveolar lavage	<ul style="list-style-type: none">• Cells collected by spraying saline water into lung alveolar spaces during endoscopy• Difficult to collect• More sensitive than sputum cytology

Main use of respiratory cytology: To look for lung cancer cells

Respiratory cytology

- Bronchial brushing and bronchio-alveolar lavage can be collected during bronchoscopy
- Lung biopsy can also be collected
→ send to histopathology lab



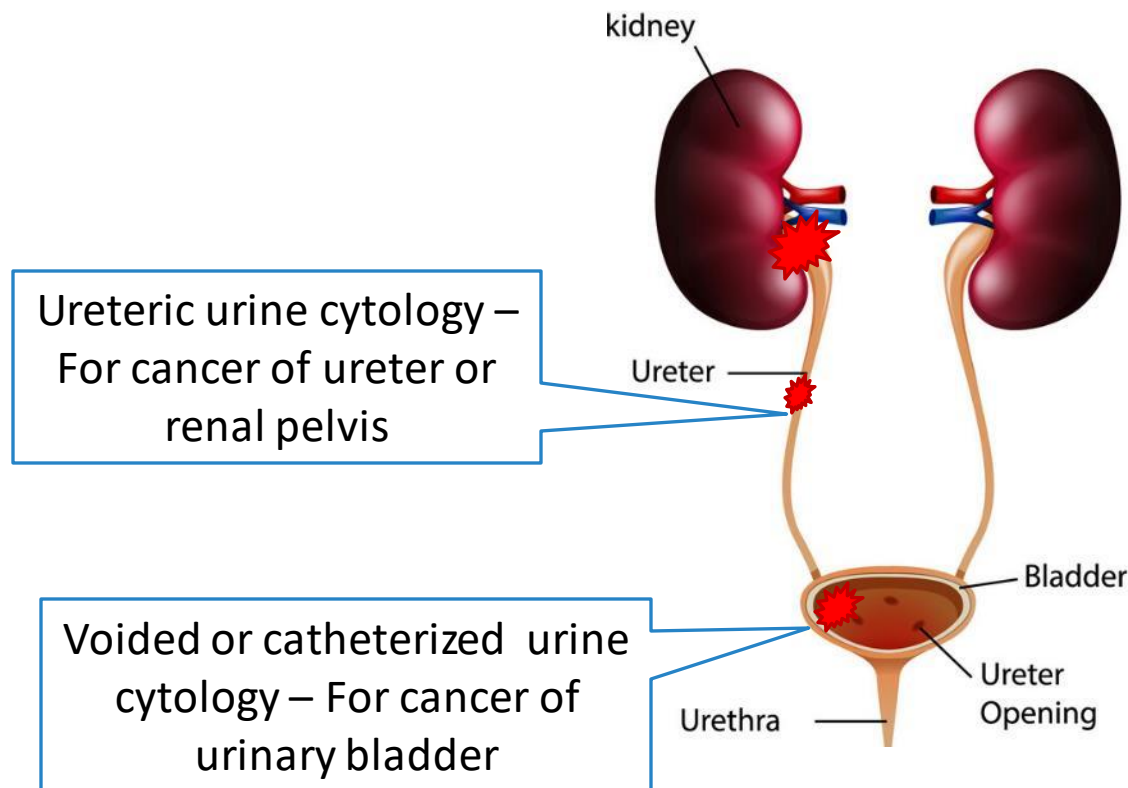
Urinary tract cytology - types

Main types	Features
Urine cytology	<ul style="list-style-type: none">• Cells from patient's urine• Can also be collected during bladder catheterization• Easy to collect• Sensitivity low
Ureteric urine	<ul style="list-style-type: none">• Urine from the upper urinary tract (left or right ureter)• Can be obtained during endoscopy• For diagnosis of cancer in ureter or renal pelvis

Main use of urine cytology:

1. To look for urinary bladder cancer cells
2. To look for cancer cells in the ureters

Upper vs. lower urinary tract cytology



Body cavity fluid cytology

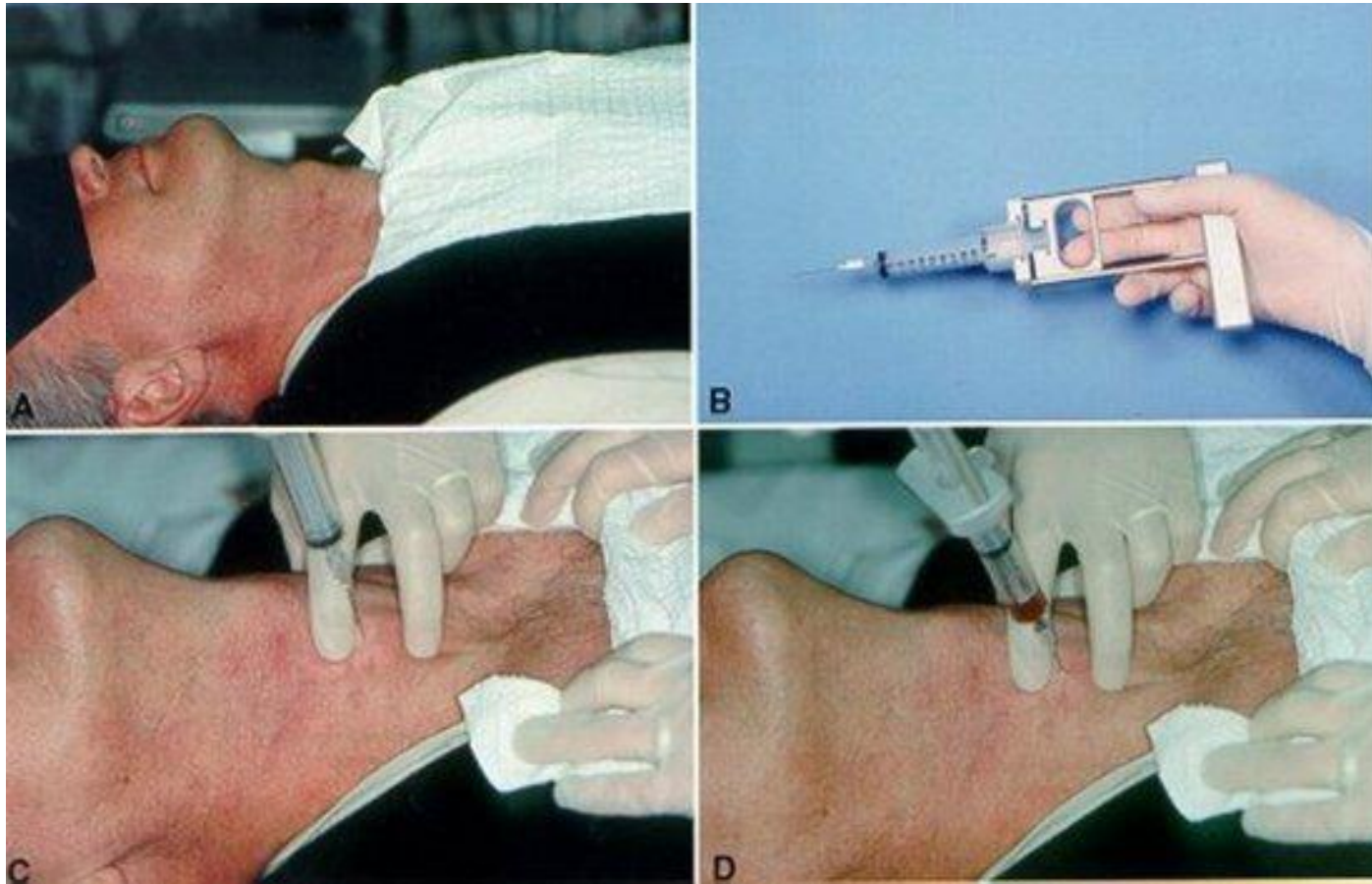
Main types	Features
Pleural fluid	<ul style="list-style-type: none">• Cells from patient's pleural effusion• Collected during drainage of the pleural effusion• To look for metastatic cancer cells in pleural cavity
Ascitic fluid	<ul style="list-style-type: none">• Cells from patient's ascites• Collected during drainage of the ascites• To look for metastatic cancer cells in peritoneal cavity
Cerebrospinal fluid	<ul style="list-style-type: none">• Cells from patient's cerebrospinal fluid• Collected during lumbar puncture• To look to cancer cells or microorganisms

Main use of urine cytology:
- To look for metastatic cancer cells

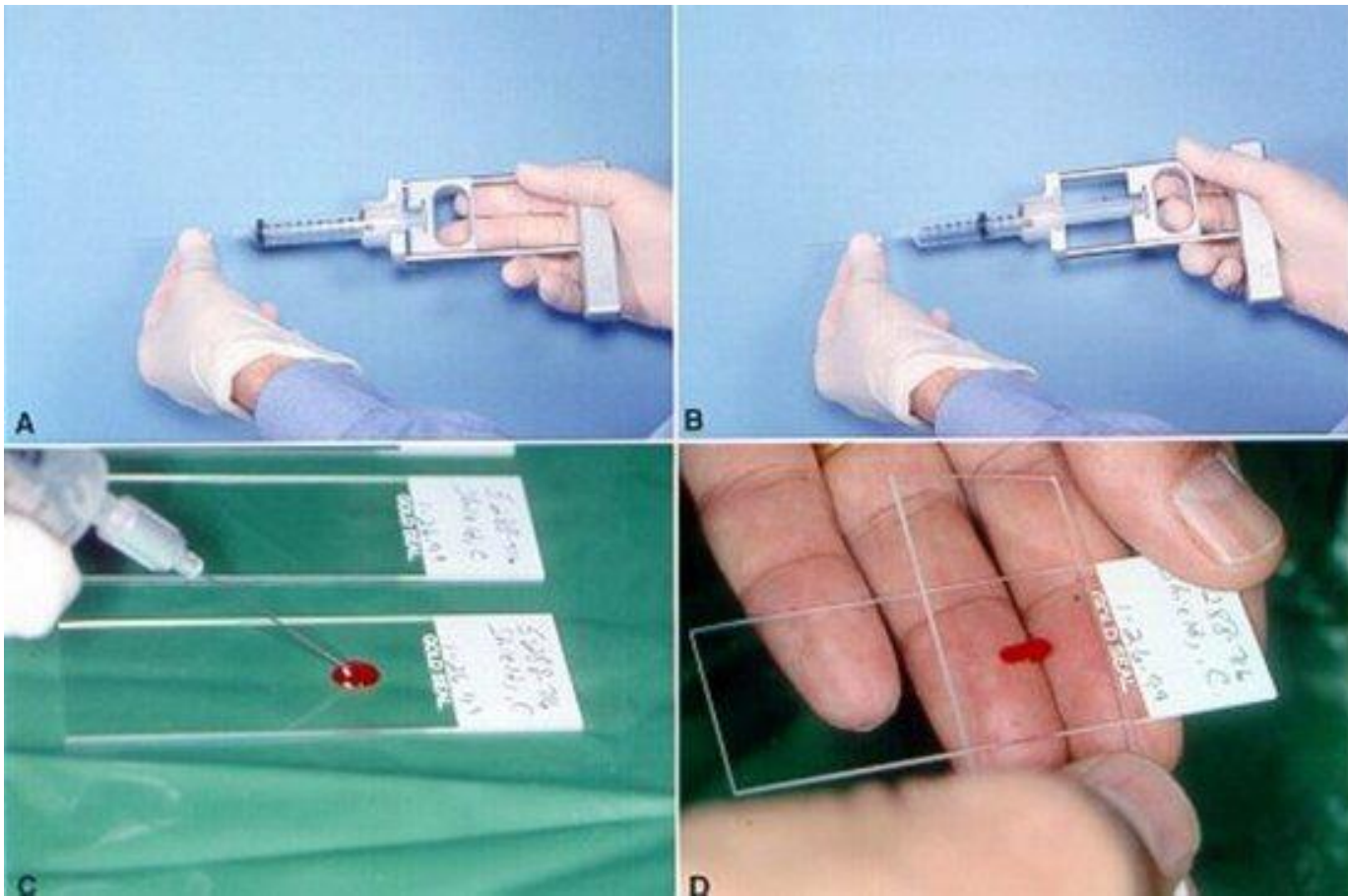
Aspiration cytology

- Cells aspirated from a tumour/lesion by fine needle
- Cells then ejected onto microscope slide and spread thinly
- The slide are stained and viewed under microscope
- Processing time faster than histology specimens

Aspiration cytology



Aspiration cytology



Body sites suitable for fine needle aspiration cytology

Superficial lesions (Can be done in clinic)

- Salivary gland
- Thyroid
- Breast lumps
- Neck lymph nodes
- Axillary lymph nodes
- Groin lymph nodes
- Other skin lumps

Deep-seated lesions (Need CT scan or endoscope)

- Intra-thoracic lesions
 - Lung tumours (via CT scan)
 - Mediastinal lymph nodes (via endoscopy or CT scan)
- Intra-abdominal
 - Liver tumours (rarely done)
 - Pancreas tumours (via endoscopy)
 - Retroperitoneal lymph nodes (via CT scan)

How non-cervical cytology specimens are usually reported

Diagnosis	Meaning
Inadequate for diagnosis	<ul style="list-style-type: none">• Not enough cells in the specimen for diagnosis• Should collect another sample from the patient
Negative for malignant cells	<ul style="list-style-type: none">• No cancer cell seen in the specimen
Atypical cells seen	<ul style="list-style-type: none">• A few mildly abnormal cells seen, but significance uncertain• Can observe the patient for a while, but can consider collecting another sample if clinically looks like cancer, or do additional investigations
Suspicious of malignancy	<ul style="list-style-type: none">• A few very abnormal cells seen, which may be cancer• Diagnosis difficult to confirm because not enough cells• Consider collecting another sample from the patient, or consider biopsy instead of cytology
Carcinoma cells present	<ul style="list-style-type: none">• Many cancer cells seen• Report may mention specific type of cancer if possible (e.g. squamous cell carcinoma, adenocarcinoma, etc.)

Summary

After this lecture, you should be able to:

- Name the major diagnostic laboratories in a hospital
 - Anatomical Pathology
 - Haematology
 - Clinical Biochemistry
 - Medical Microbiology
 - Immunology
- Aware of the basic workflow of ordering laboratory investigations
- Name the major services provided by the Anatomical Pathology Laboratory
 - Histopathology: For biopsy and large organ resections
 - Cytology: For cervical cancer screening, exfoliated cells from fluids, and fine needle aspiration