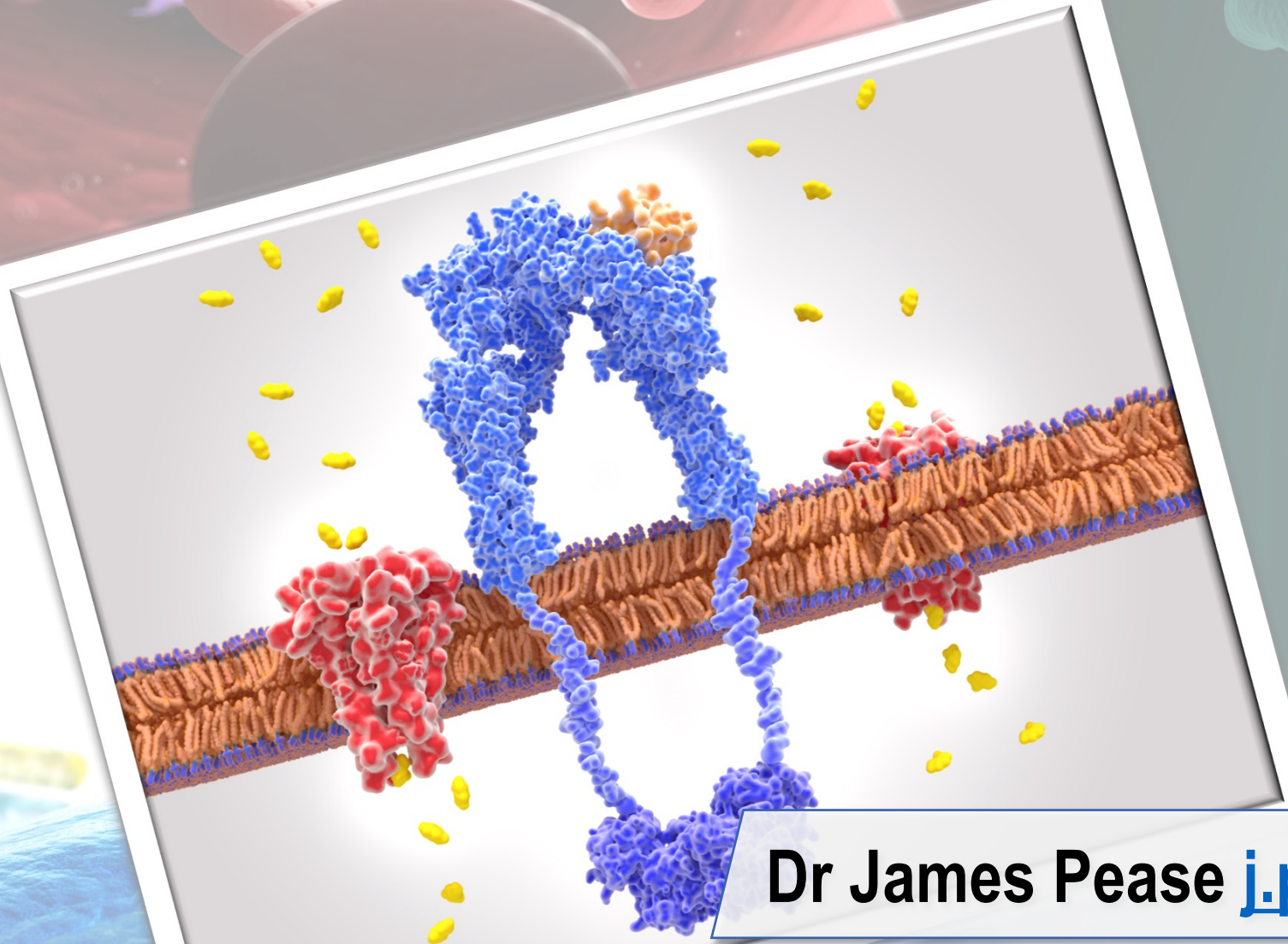
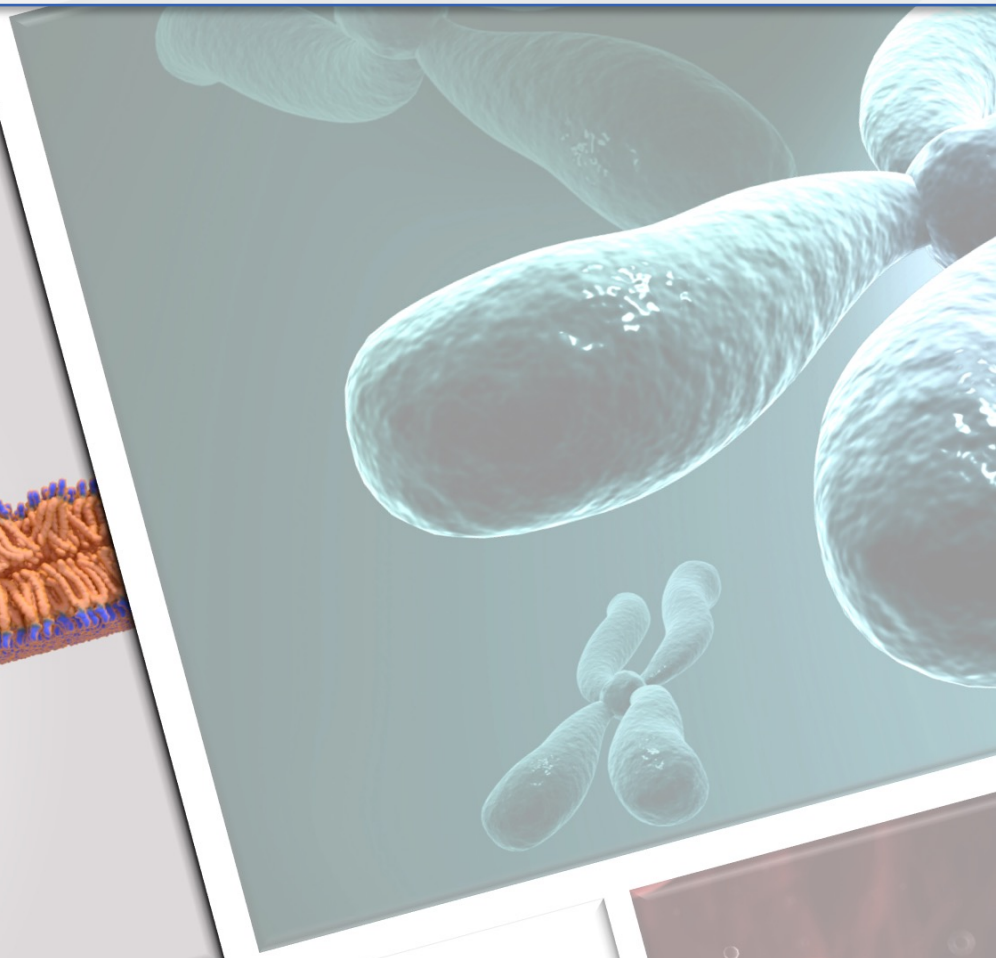
Imperial College London

Histopathology





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Session Plan

Part 1

- Histopathology
  - Biopsies
  - Resection specimens
  - Frozen sections
- Cytopathology
  - Smears
  - Fine needle aspirates

Part 2

- Conjugation of antibodies
  - Enzymes
  - Fluorescent markers
  - Magnetic beads
- Use of antibodies in diagnosis
  - Immunohistochemistry
  - ELISA
  - Flow cytometry

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Histopathology v cytopathology

A **histopathologist** deals with **tissues**.

She/he will examine sections, noting the architecture of the tissue and asking what it tells us about a particular condition.

A **cytopathologist** deals with **cells.**

They are often are the individuals responsible for taking the cells from the patient, preparing them for examination and then delivering their expert diagnosis on the cell sample

Histopathology

A **histopathologist** deals with **tissues**.

Architecture? Likely medical condition?

Useful for diagnosis and to determine the efficacy of a treatment.

A histopathologist works a variety of tissue samples:

- Biopsies
- Resection specimens
- Frozen sections
- Post-mortems

Real-time examination can inform the surgical process.

1-POM-1-10: Histopathology and cytopathology: Recall situations where histopathology and cytopathology might be used as a diagnostic method, and summarise the main steps involved in processing specimens.

Biopsies

A biopsy embedded in paraffin wax ready to be cut into sections

Biopsies are **small sections** of tissues removed from a patient.

Preserved in a formalin solution which cross-linking proteins and **fixes** the tissue.

Embedded in **paraffin wax** to allow very thin sections (2-3µm thick) to be using a microtome.

Mounted on a microscope slide for further preparation prior to analysis.

Is the tissue normal? Is the tissue inflamed? What is the likely cause? (**1-POM-1-9 Inflammation**)

Is the tissue cancerous and, if it is, what type of cancer is it? (**1-POM-1-7 Cancer**)

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Resection specimens

Resections are taken from tissue that has been removed as part of a surgical procedure and can be processed as for a biopsy.

Resections are used primarily to look at the stage the disease (**1-POM-1-7 Cancer**).

Has the cancer penetrated the bowel wall, spread to the lymph nodes or the liver?

Has all the cancerous tissue been removed or is chemotherapy also needed?

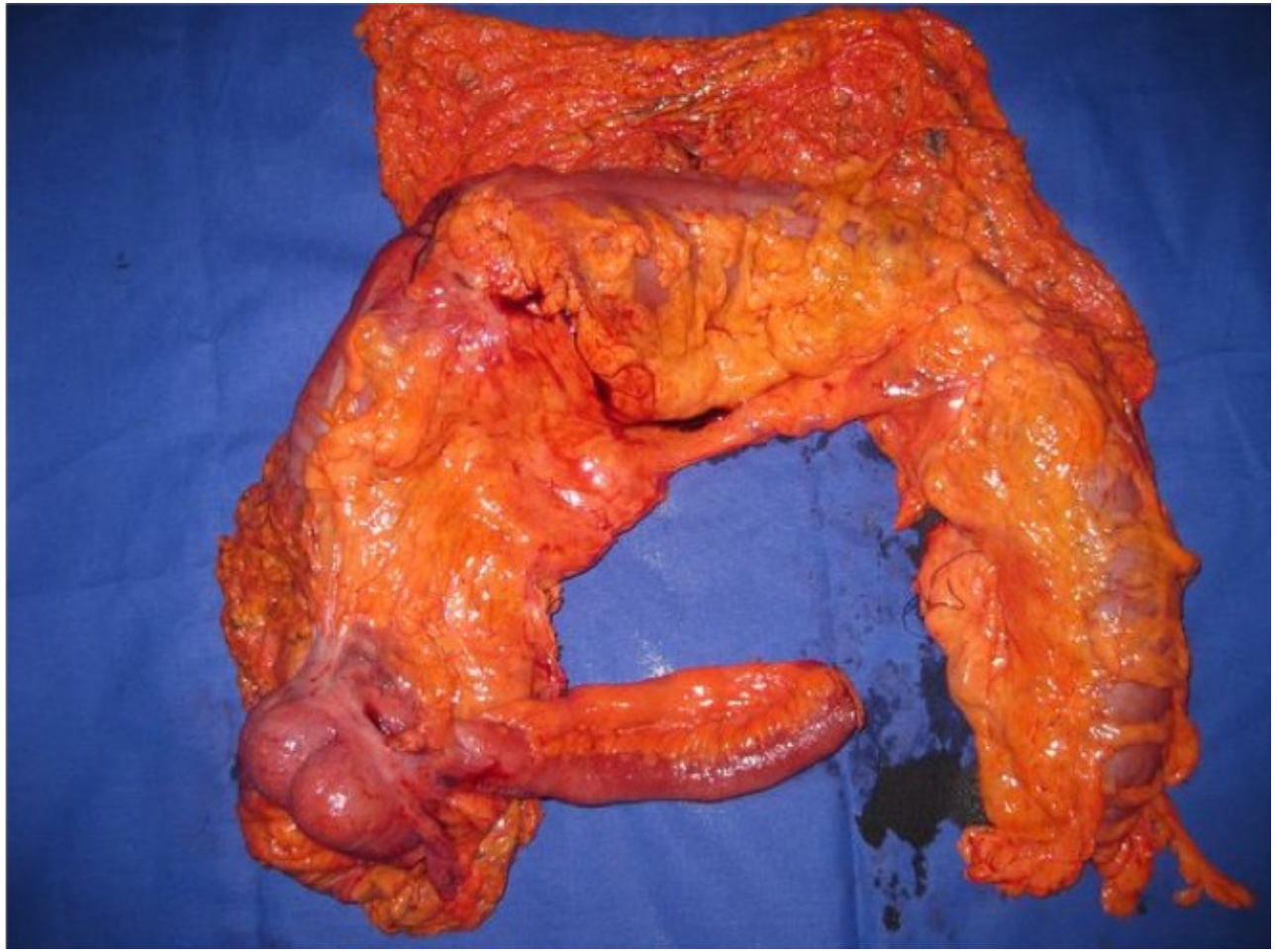

Figure: Resection tissue taken for pathological analysis

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Frozen sections

Figure: Preparation of a frozen section

Frozen sections - taken during surgical procedures and examined in **real time** during the operation.

The freshly taken tissue is frozen by a **cryostat,** cut, mounted on slides and stained as for biopsies.

Provides diagnosis in minutes which can be relayed back to the surgeon to inform the surgery.

For example:

- Is the tissue cancerous?
- Has all the cancerous tissue been removed?
- Is there another pathological process going on?

Timescales

Key considerations are the relative timescales for a result from the histopathology lab to reach the clinician:

- Frozen section: **30 minutes**
- Biopsies: **2-3 days**
- Resection specimen: **5-7 days**

Cytopathology

Human pap smear stained with H&E and showing the presence of chlamydia in the vacuoles.
Source; National Cancer Institute.

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Fine needle aspirates

A patient undergoing a fine needle aspiration.

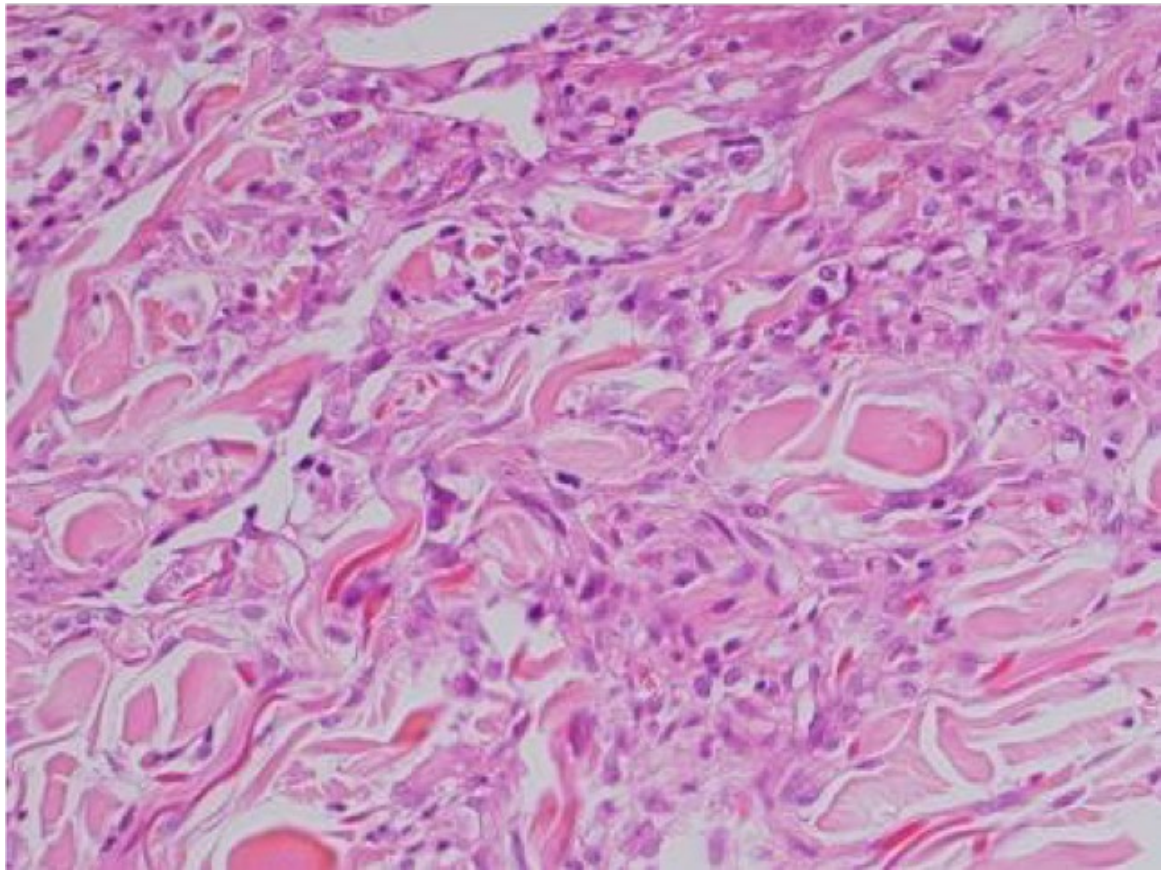
Advantages

Can penetrate relatively inaccessible tissues e.g. thyroid nodule and assess a condition without surgery.

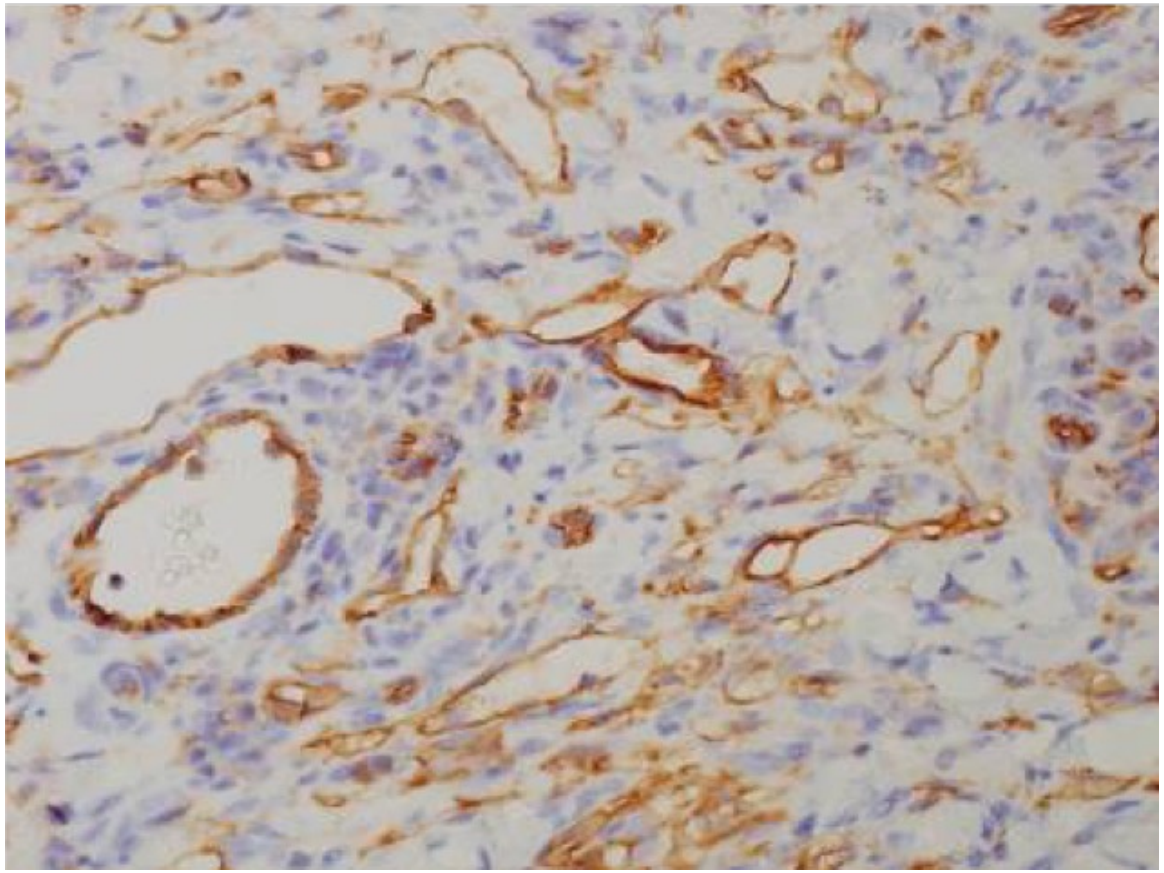
Disadvantages

Looks at cells in isolation – no information regarding tissue architecture.

Histopathology and cytopathology are a powerful combination



Skin biopsy taken from an individual with Kaposi's sarcoma.



CD31 immunostaining of a skin biopsy.

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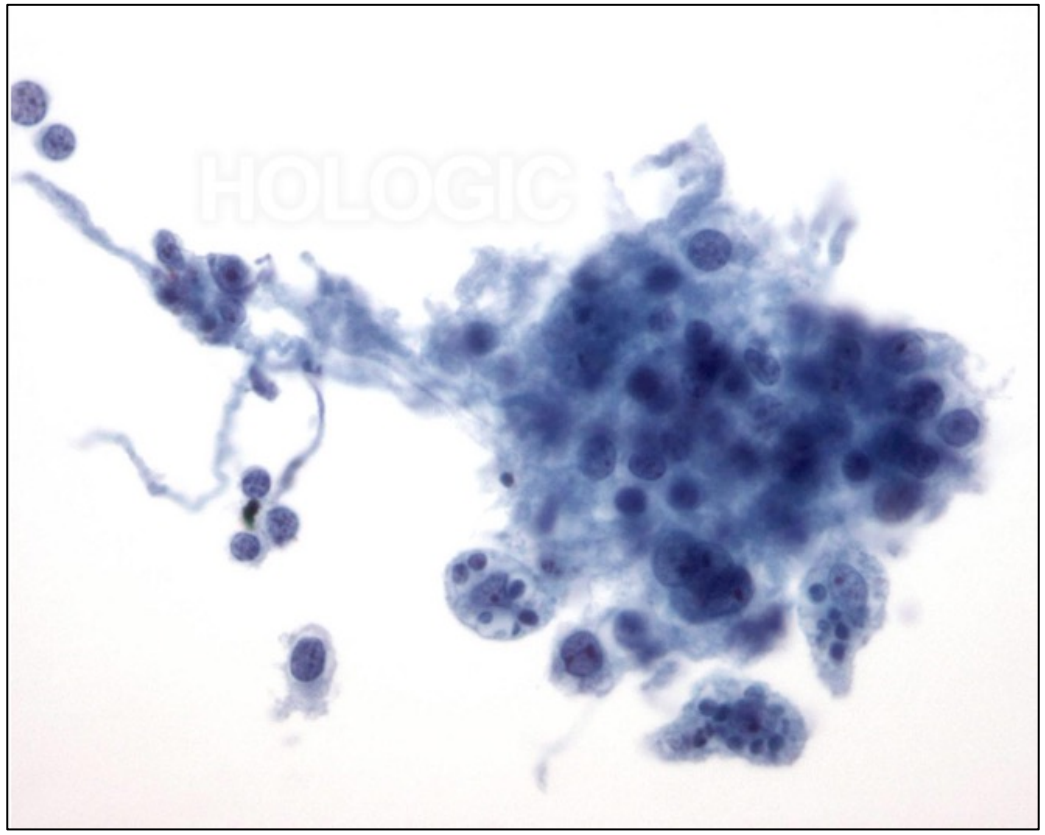
Histopathology and cytopathology are a powerful combination



Cytopathological analysis of a fine needle aspirate

Diagnosis of reactive lymphadenopathy

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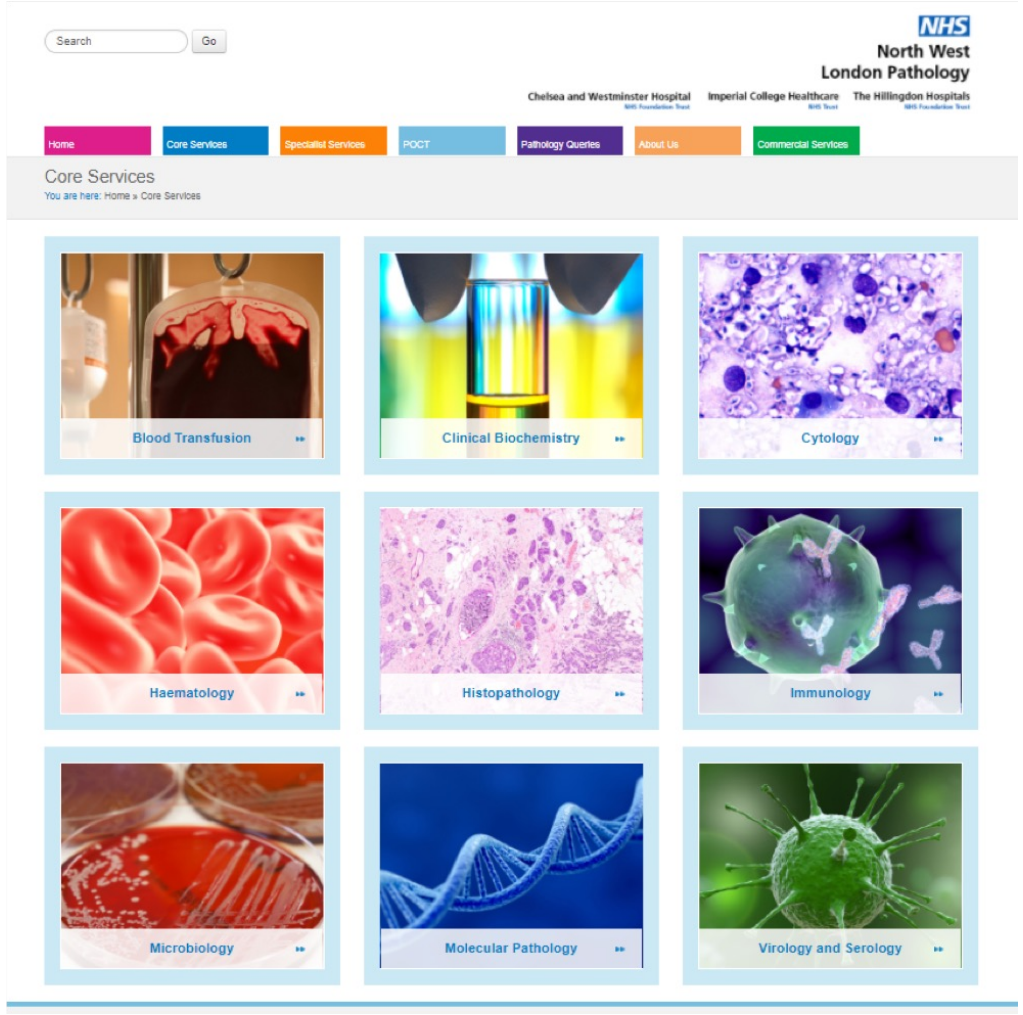
Antibodies –versatile tools for diagnosis



The immunology labs offer 86 different tests of which 53 are used to detect the levels of specific antibodies circulating in patients.

e.g. ds DNA

- systemic lupus erythematosus (SLE)
- Sjögren's syndrome
- rheumatoid arthritis

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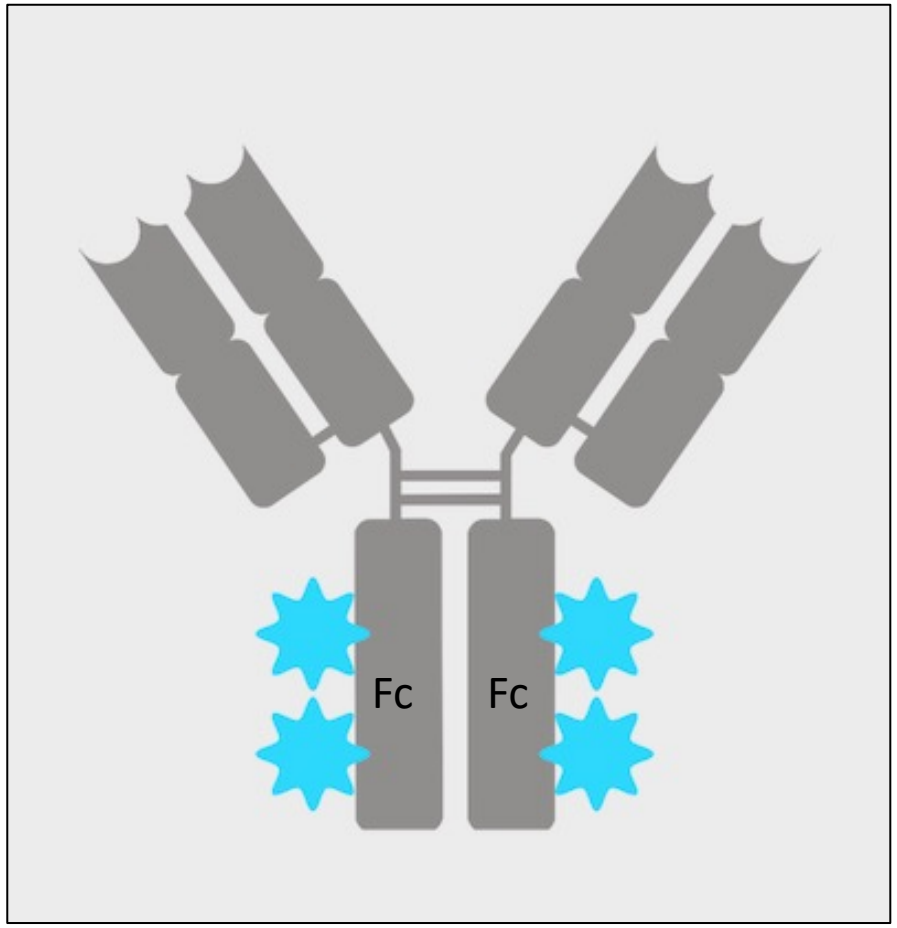
Antibody conjugates



Conjugations

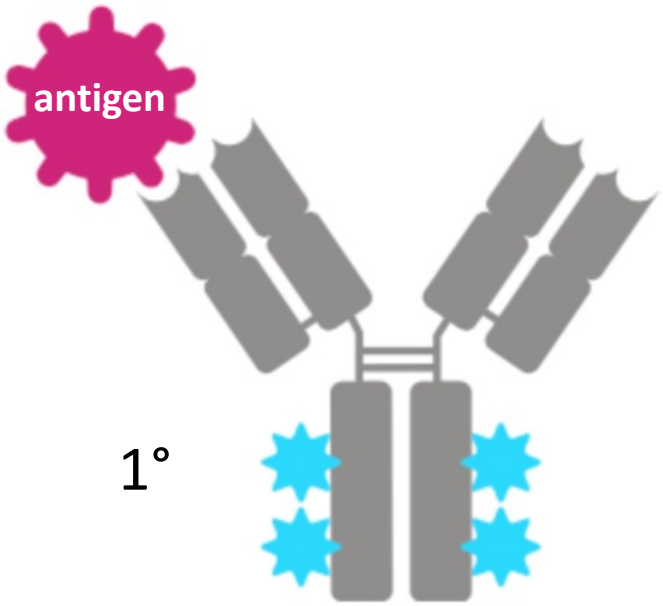
Enzymes: e.g. peroxidase, alkaline phosphatase.

Fluorescent probes. These can allow the rapid measurement of the levels of molecules within a sample.

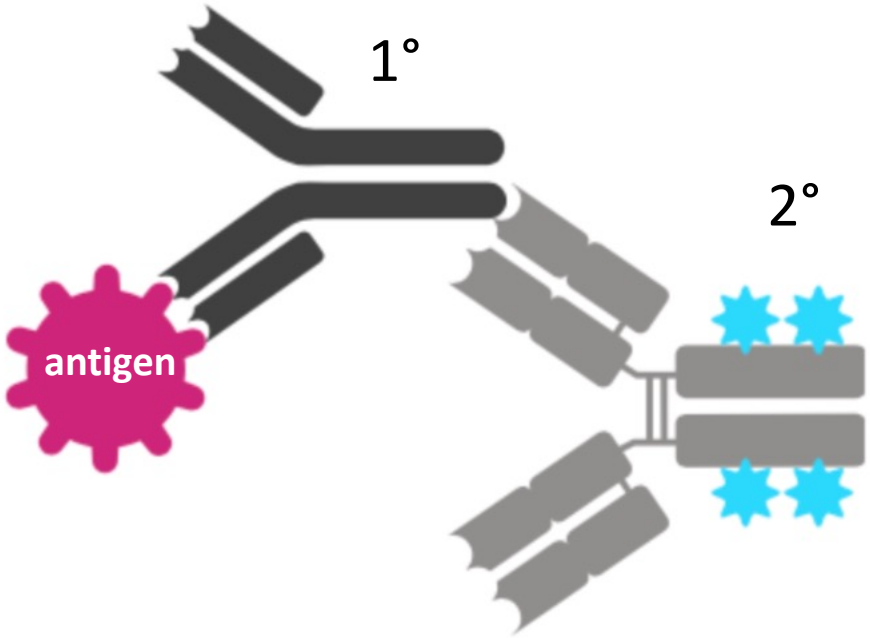
Magnetic beads: e.g. the purification of cell types.

Drugs: e.g. Kadcyla, an anti-**HER2** antibody linked to the cytotoxic chemical **emtansine.**

Antibodies as diagnostic tools



direct detection



indirect detection

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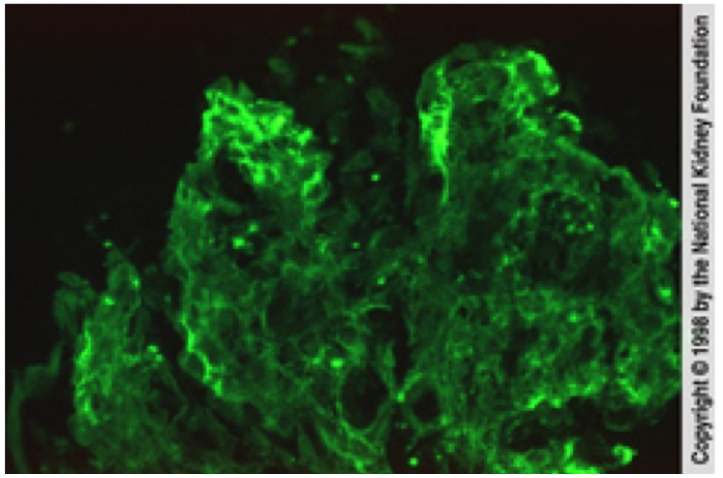
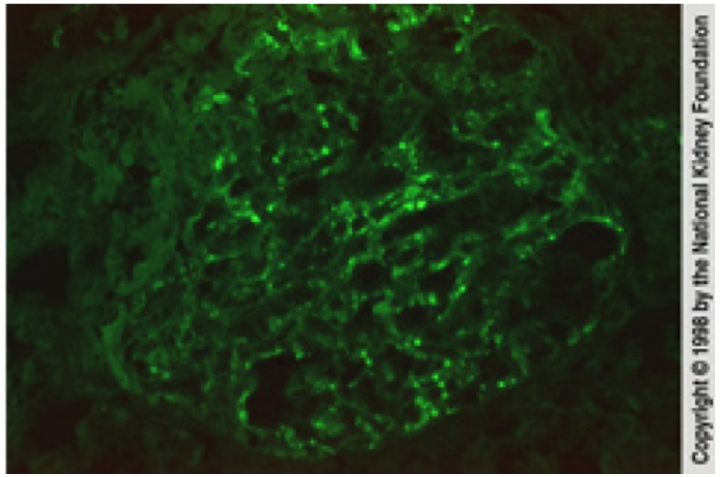
Use of manufactured antibodies

- Blood group serology (**Haem -Blood transfusion**).
- Immunoassays
  e.g. detection of hormones
  circulating antibodies/antigens.
- Immunodiagnosis
  e.g. infectious diseases
  antibody levels
  IgE (**Immno - Hypersensitivity**).

kidney biopsy



IgG-antigen



Complement C3

ELISA (Enzyme linked immunosorbent assay)

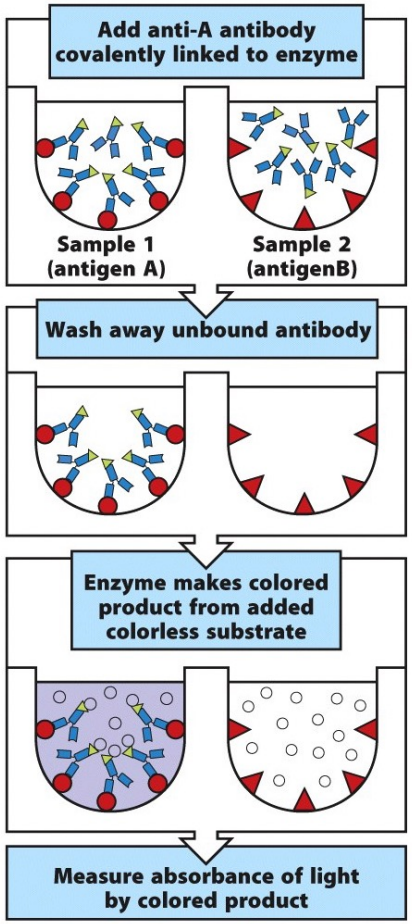


Figure A.6 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

ELISA

Enzyme
Linked
ImmunoSorbent
Assay

- Clinical samples (e.g. adheres to a plastic plate
- Probe with specific antibody raised against the molecule of interest.
- Enzyme conjugation generates a coloured product.
- Reference to standard curve (**MBC - Haemoglobin**) determine precise concentrations of the molecule in the sample.

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Flow cytometry

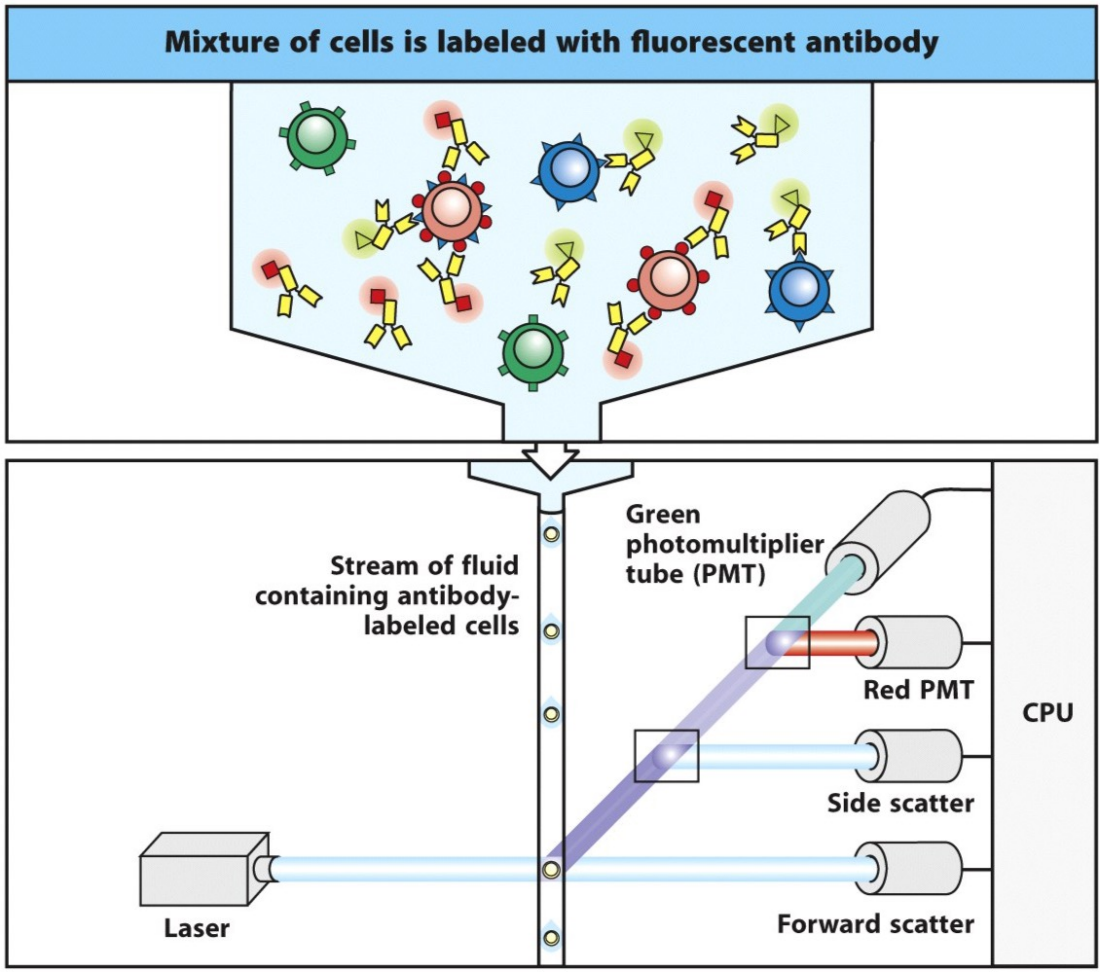

Figure A.26 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Cells labelled with **differently** conjugated Abs

Run as a stream of single cells through a laser beam (s).

Colour of light emitted and the forward or side scatter of the laser beam denotes the **identity** of the cell surface molecules expressed and the **size** and **granularity** of the cells.

- anti-$CD3^+$ T cells – pan T cell marker
- anti-$CD4^+$ T cells – T helper/cells
- anti–$CD8^+$ T cells – cytotoxic T cells
- anti–$CD19^+$ B cells
- anti–$CD56^+$ Natural Killer (NK) Cells

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With many thanks to Dr Keith Gould and Professor Robert Goldin