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1. Diagnostic Techniques Used in Pathology;
2. Histopathology; this is the diagnosis and study of diseases of the tissues, and involves examining tissues and/or cells under a microscope. Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen has been processed and histological sections have been processed and histological sections have been placed onto glass slides.

Histological procedures aim to provide good quality sections that can be used for a light microscopic evaluation of human or animal tissue changes in either spontaneous or induced diseases. The section of tissue collected from large sample, this process is called Grossing or Cut up. There are three main types of specimen received by the pathology laboratory;

1. Larger specimens include whole organs or parts thereof, which are removed during surgical operations. Examples include a uterus after a hysterectomy, the larger bowel after a colectomy or tonsils after a tonsillectomy.
2. Pieces of tissue rather than whole organs are removed as biopsies, which often require smaller surgical procedures that can be performed whilst the patient is still awake but sedated. Biopsies include excision biopsies; in which tissue is removed with a scalpel (e.g a skin excision for a suspicious mole) or a core biopsy; in which a needle is inserted into a suspicious mass to remove a slither or core of tissue that can be examined under the microscope (e.g to investigate a breast lump).
3. Fluid and very small pieces of tissue (individual cells rather than groups of cells, e.g within fluid from around the lung) can be obtained through a fine needle aspiration (FNA). This is performed using a thinner needle than the needle used in core biopsy, but with a similar technique.

The tissue is then prepared for viewing under a microscope using either chemical fixation or frozen section.

1. Chemical fixation; in addition to formalin, other chemical fixatives have been used. But, with the advent of immunohistochemistry (HIC) staining and diagnostic molecular pathology testing on these specimen samples, formalin has a standard chemical fixatives in human diagnostic histopathology.
2. Frozen section processing; the second method of histology processing is called frozen section processing. This is a highly technical scientific method performed by a trained Histoscientist. In this method, the tissue is frozen and sliced thinly using a microtome mounted in a below-freezing refrigeration device called the Cryostat. The thin frozen sections are mounted on a glass slide, fixed immediately and briefly in liquid fixative, and stained using the similar staining techniques as traditional wax embedded sections.
3. Cytopathology; is a branch of pathology that studies and diagnoses diseases on the cellular level. Cytopathology is the study of cells from various body sites to determine the cause or nature of disease. Cytopathologic methods include;
4. Exfoliative cytology; refers to the examination of cells that are shed spontaneously into body fluids or secretions. Examples include; sputum, cerebrospinal fluid, urine, effusions in body cavities (pleura, pericardium, peritoneum), nipple discharge and vaginal discharge.
5. Intervention cytology; in intervention cytology, the pathologist intervenes into the body for sample collection. Such as;
6. Fine needle aspiration cytology (FNAC); in FNAC, cells are obtained by aspirating the diseased organ using a very thin needle under negative pressure. Virtually any organ or tissue can be sampled by fine-needle aspiration. Deep organs, such as the lung, mediastinum, liver, pancreas, kidney, adrenal gland, and retroperitoneum are aspired with guidance by fluoroscopy, ultrasound or CT scan.
7. Abrasive cytology; refers to methods by which cells are dislodged by various tools from body surfaces (skin, mucous membranes, and serous membrane). Example; preparation of cervical smears with a spatula or a small brush to detect cancer of the uterine cervix at an early stage. Such cervical smears are called Pap smears, can significantly reduce the mortality from cervical cancer.
8. Sediment cytology; the sample is collected from the fixative that was used for processing the biopsy or autopsy specimen. The fixative is mixed properly and taken into a centrifuge tube and is centrifuged. The sediment is used for smearing. these sediments are the cells that are shed by the autopsy and biopsy specimen during processing.
9. Imprint cytology; is a preparation wherein the tissue of interest touches a glass slide, leaving behind its imprint in the form of cells on the slide. The imprint can subsequently be stained and studied.
10. Hematopathology; is the study of diseases and disorders affecting and found in blood cells and their precursors in the bone marrow, their production, and any organs and tissues involved in hematopoiesis, such as bone marrow, the spleen, and the thymus. Diagnosis and treatment of diseases such as Leukemia and Lymphoma often deal with hematopathology; techniques and technologies include flow cytometry studies and immunohistochemistry. There are six laboratories of division of hematopathology are;
11. Cell kinetics; uses multicolour flow cytometry to immunophenotypically detect and characterize neoplastic hematolymphoid cells.
12. Metabolic hematology; detects abnormal hemoglobins (examples; sickle cell disease), red blood cell enzyme deficiencies and cytoskeletal abnormalities (example; hereditary spherocytosis) and also screens for and characterizes thalassemias.
13. Molecular hematopathology; analyses RNA and DNA to detect and quantify genetic abnormalities useful in the diagnosis and monitoring of hematologic malignancies.
14. Hematopathology morphology; performs complete blood counts (CBC) and analyses cellular morphology in peripheral blood and some body fluids.
15. Special coagulation; performs testing to aid in the diagnosis and treatment of patients who may have bleeding or clotting disorders.
16. Special DNA coagulation; concentrates on the identification of genetic abnormalities associated with hereditary bleeding and clotting disorders.
17. Autopsy; an autopsy (post-mortem examination, necropsy, or autopsia cadaverum) is a surgical procedure that consists of a thorough examination of a corpse by dissection to determine the cause, mode, and manner of death or to evaluate any disease or injury that may be present for research or educational purposes. The purpose of Autopsies are:
18. Determines if death was natural or unnatural
19. Injury source and extent on the corpse
20. Manner of death must be determined
21. Retain relevant organs
22. Time of death
23. Establish identity of the deceased.

Types of autopsy;

1. Medio-legal or Forensic; seeks to find the cause and manner of death and to identify the decedent. They are generally performed, as prescribed by applicable law, in cases of violent, suspicious or sudden deaths, deaths without medical assistance or during surgical procedures. A forensic may include obtaining biological specimens from the deceased for toxicological testing, including stomach contents. Toxicology tests may reveal presence of one or more chemicals (poisons) and their quantity.
2. Clinical or Pathological autopsies; are performed to diagnose a particular disease or for research purposes. They aim to determine, clarify, or confirm medical diagnoses that remained unknown or unclear prior to the patient’s death. Clinical autopsies serves two major purposes;
3. They are performed to gain insight into **pathological processes** and determine what factors contributed to the patient’s death. for example, material for infectious disease testing can be collected during an autopsy.
4. Autopsies are also performed to ensure the standard of care at hospitals. It can yield insights into how patient deaths can be prevented in the future.
5. Anatomical or Academic autopsies; are performed by students of anatomy and other medical courses for study purposes.
6. Virtual or Medical imaging autopsies; are performed utilizing imaging technology only, primarily magnetic resonance imaging (MRI) and computed tomography (CT).
7. Cytogenetics; is essentially a branch of genetics, but is also a part of cell biology/cytology (a subdivision of human anatomy), that is concerned with how the chromosomes relate to cell behavior, particularly to their behavior during mitosis and meiosis. This method in which inherited chromosomal abnormalities in the germ cells or acquired chromosomal abnormalities in somatic cells are investigated using techniques of molecular biology. Techniques used include Karyotyping analysis of G-banded chromosomes, molecular cytogenetics etc.

Techniques:

1. Karyotyping; the routine chromosome analysis (Karyotyping) refers to analysis of metaphase chromosomes which have been banded using trypsin followed by Giemsa, Leishmanns or a mixture of the two. This creates unique banding patterns on the chromosomes. The molecular mechanism and reason of these patterns is unknown, although it likely related to replication timing and chromatin packing.
2. Fluorescent in situ hybridization (FISH); refers to using fluorescently labeled probe to hybridize to cytogenetic cell preparations. In addition to standard preparations FISH can also be performed on:
3. Bone marrow smears
4. Blood smears
5. Paraffin embedded tissue preparations
6. Enzymatically dissociated tissue samples
7. Uncultured bone marrow
8. Uncultured amniocytes
9. Cytospin preparation.
10. Comparative genomic hybridization (CGH) and array comparative genomic hybridization (CGH); CGH is a method of molecular cytogenetic testing that detects chromosomal copy number variants (portions of the genome where sections of genes are doubled or tripled) without the need for cell culturing. It was first developed to identify such changes in tumors.

CGH uses 2 genomes; the test sample and a control, both of which are fluorescently labelled to differentiate between the two. The two samples are denatured and mixed together, allowing hybridization of metaphase chromosomes. The intensity of the fluorescent signal of the labelled test DNA relative to the control DNA can then be plotted along each chromosome, which shows the loss or gain of genetic material and allows identification of any copy number variants.

CGH differs from other methods of cytogenetic testing in that it does not rely on a specific target, nor does it require previous knowledge of the region under examination. Instead, CGH can quickly scan a whole genome for chromosomal imbalances and it is useful in cases where the diagnosis is not known. One limitation of CGH is the size of the genetic alteration that it can identify the resolution of CGH is poor at approximately 5–10 mega-bases.

CGH utilizes a similar technique as CGH but it provides a much higher resolution by using microarrays. Small sections of DNA are used as targets for analysis; these sections are immobilized on solid support, which anchors DNA to a spot without altering the protein. As in CGH, the sample DNA and control are fluorescently labelled to differentiate them.

The samples are then mixed and added to the microarray where they compete to bind to the probes on the microarray. The strength of the different fluorescent signals can be assessed and any small gains or losses within the DNA are identified. A disadvantage of CGH is that it cannot detect balanced chromosomal structural changes such as balanced translocations or inversions.

Benefits of cytogenetic testing.

Cytogenetic testing can offer diagnosis and help with the long-term management of relevant diseases. It also allows genetic counseling for the patient or their parents about the related risk in any future pregnancies and, in some cases, guides the geneticist as to whether to test other family members.

Disadvantage of cytogenetic testing.

Cytogenetic testing is limited by its resolution. The different methods can identify small gains and losses of genetic material, as well as larger translocations, but they do not allow testing for single nucleotide variations that could contribute to the patient’s condition. There is also the possibility that cytogenetic testing will identify other chromosomal changes that are not necessarily related to the patient’s condition.

1. Cellular adaptation precedes cell death;

Cellular adaptation is the ability of cells respond to various types of stimuli and adverse environmental changes. These adaptations include; Hypertrophy (enlargement of individual cells), Hyperplasia (increase in the number of cells), Atrophy (reduction in the number and size of cells), Metaplasia (transformation of one epithelium to another) and Dysplasia (disordered growth of cells). Tissues adapt differently depending on the replicative characteristics of the cells that make up the tissue. For example, labile tissue such as the skin can rapidly replicate and therefore can also regenerate after injury whereas permanent tissue such as neural and cardiac tissue cannot regenerate after injury. Its cells are not able to adapt to the adverse environmental changes. Cellular adaptation could be normal (physiological) or abnormal (pathological).

When cells are injured, one or two patterns will gradually occur; reversible cell injury leading to adaptation of the cells and tissues, or irreversible cell injury leading to cell death and tissue damage. Injured cells may accumulate materials including fat, cholesterol, protein, glycogen or pigment. When cells are irreversibly injured and dying, specific nuclear changes may be visible including pyknosis, karyrrhexis and karyolysis. If large number of cells dies, tissue necrosis may occur. Observable patterns of necrosis include; coagulative, liquefactive, fibrinous, gummatous, fat, gangrene and caseous necrosis.



