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**17/MHS02/069**

**NURSING DEPARTMENT**

**300 LEVEL**

**CELLULAR PATHOLOGY ASSIGNMENT**

**DIAGNOSTIC TECHNIQUES USED IN PATHOLOGY.**

1. **URINALYSIS**: A urinalysis is a test of your urine. A urinalysis is used to detect and manage a wide range of disorders, such as urinary tract infections, kidney disease and diabetes. A urinalysis involves checking the appearance, concentration and content of urine. Abnormal urinalysis results may point to a disease or illness. For example, a urinary tract infection can make urine look cloudy instead of clear. Increased levels of protein in urine can be a sign of kidney disease. Unusual urinalysis results often require more testing to uncover the source of the problem.

REASONS FOR URINALYSIS.

1. To check your overall health.
2. To diagnose a medical condition.
3. To monitor a medical condition.

PREPARATION.

If your urine is being tested only for a urinalysis, you can eat and drink normally before the test. Many drugs, including nonprescription medications and supplements, can affect the results of a urinalysis. Before a urinalysis, tell your doctor about any medications, vitamins or other supplements you're taking.

PROCEDURE.

Depending on your situation, you may collect a urine sample at home or at your doctor's office. Your doctor will provide a container for the urine sample. You may be asked to collect the sample first thing in the morning because at that time your urine is more concentrated, and abnormal results may be more obvious.

To get the most accurate results, the sample may need to be collected midstream, using a clean-catch method. This method involves the following steps:

* Cleanse the urinary opening. Women should spread their labia and clean from front to back. Men should wipe the tip of the penis.
* Begin to urinate into the toilet.
* Pass the collection container into your urine stream.
* Urinate at least 1 to 2 ounces (30 to 59 milliliters) into the collection container.
* Finish urinating into the toilet.
* Deliver the sample as directed by your doctor.
* If you can't deliver the sample to the designated area within 60 minutes of collection, refrigerate the sample, unless you've been instructed otherwise by your doctor.
* In some cases, your doctor may insert a thin, flexible tube (catheter) through the urinary tract opening and into the bladder to collect the urine sample.
* The urine sample is sent to a lab for analysis. You can return to your usual activities immediately.

For a urinalysis, your urine sample is evaluated in three ways: visual exam, dipstick test and microscopic exam.

1. Visual exam

A lab technician examines the urine's appearance. Urine is typically clear. Cloudiness or an unusual odor may indicate a problem, such as an infection.Blood in the urine may make it look red or brown. Urine color can be influenced by what you've just eaten. For example, beets may add a red tint to your urine.

1. Dipstick test

A dipstick — a thin, plastic stick with strips of chemicals on it — is placed in the urine to detect abnormalities. The chemical strips change color if certain substances are present or if their levels are above normal. A dipstick test checks for:

* Acidity (pH): The pH level indicates the amount of acid in urine. Abnormal pH levels may indicate a kidney or urinary tract disorder.
* Concentration: A measure of concentration, or specific gravity, shows how concentrated particles are in your urine. A higher than normal concentration often is a result of not drinking enough fluids.
* Protein: Low levels of protein in urine are normal. Small increases in protein in urine usually aren't a cause for concern, but larger amounts may indicate a kidney problem.
* Sugar: Normally the amount of sugar (glucose) in urine is too low to be detected. Any detection of sugar on this test usually calls for follow-up testing for diabetes.
* Ketones: As with sugar, any amount of ketones detected in your urine could be a sign of diabetes and requires follow-up testing.
* Bilirubin: Bilirubin is a product of red blood cell breakdown. Normally, bilirubin is carried in the blood and passes into your liver, where it's removed and becomes part of bile. Bilirubin in your urine may indicate liver damage or disease.
* Evidence of infection: If either nitrites or leukocyte esterase — a product of white blood cells — is detected in your urine, it may be a sign of a urinary tract infection.
* Blood: Blood in your urine requires additional testing — it may be a sign of kidney damage, infection, kidney or bladder stones, kidney or bladder cancer, or blood disorders.
1. Microscopic exam

During this exam, several drops of urine are viewed with a microscope. If any of the following are observed in above-average levels, additional testing may be necessary:

* White blood cells (leukocytes) may be a sign of an infection.
* Red blood cells (erythrocytes) may be a sign of kidney disease, a blood disorder or another underlying medical condition, such as bladder cancer.
* Bacteria or yeasts may indicate an infection.
* Casts — tube-shaped proteins — may form as a result of kidney disorders.
* Crystals that form from chemicals in urine may be a sign of kidney stones.

A urinalysis alone usually doesn't provide a definite diagnosis. Depending on the reason your doctor recommended this test, abnormal results may or may not require follow-up.

1. **RADIOGRAPHY**: Diagnostic X-ray or radiography is a special method for taking pictures of areas inside the body. A machine focuses a small amount of radiation on the area of the body to be examined. The X-rays pass through the body, creating an image on film or a computer display.

Various types of diagnostic X-ray procedures are ordered for different reasons. Common procedures include:

1. Angiography: Uses an injection of contrast medium to image blood vessels in a specific part of the body. Angiograms show the function of blood vessels in the heart, lungs, kidneys, brain, arms, and legs.
2. Arthrogram: Uses an injection of contrast medium into a joint. This procedure shows injury or disease in joints, arms, and legs.
3. Upper GI (gastrointestinal) series: Uses a barium solution as a contrast medium and helps evaluate the function of the esophagus, stomach, and upper small intestine.
4. Lower GI series: Uses a barium enema to evaluate the colon and rectum.
5. Intravenous pyelogram (IVP): Uses a contrast medium injection to evaluate the kidneys, ureters, and bladder.
6. Mammography: Uses a special X-ray machine to create images of breast tissue for detection of abnormalities.

PREPARATION.

Most routine X-rays do not require patients to prepare for the exam. However, special studies, such as contrast radiography or barium enemas, require patients to follow special instructions from the doctor.

1. Patients might be asked to make dietary changes leading up to the time of the exam.
2. Patients might also be asked to leave jewelry at home, along with other metal objects that could interfere with the X-ray images.
3. Patients might be asked to avoid using deodorants, body powders, or creams on the day of the appointment.
4. They might be asked to change into a gown or smock.

At the appointment, patients will meet X-ray professionals who are specially trained to help with the procedure:

1. A radiologist is a doctor who specializes in imaging the human body.
2. A radiologic technologist is trained to operate the equipment and obtain X-ray images.
3. A radiologic nurse monitors vital signs, administers medication, and provides patient care during the procedure.

PROCEDURE.

* Once a patient has changed into the smock or gown, a technologist will escort him or her into the X-ray room to stand, sit, or lie on a table that is near an X-ray machine. An apron or shield might be placed over the patient’s body to protect sensitive organs during the exam.
* The machine will take several X-rays, and the patient might be asked to adjust position during the test. It is important to remain still during each examination.
* Patients might be asked to wait until the radiologist reviews images to be sure additional images aren’t needed. If the patient ingested a contrast medium or barium, it is important to drink plenty of liquid over the 24 to 48 hours following the scan to help pass the material.
* The radiologist will review the images and send a report to the doctor, who will notify the patient of any findings. Patients can also request to receive images on CD.

RISKS.

Some people have an allergic reaction to contrast media, which is used in certain diagnostic X-rays. Symptoms of an allergic reaction include:

1. Hives
2. Itchiness
3. Nausea
4. Shortness of breath
5. Weakness
6. **CYTOGENETIC TESTING:** Cytogenetic testing involves the analysis of cells in a sample of blood, tissue, amniotic fluid, bone marrow, or cerebrovascular fluid to identify any changes in an individual’s chromosomes.

There are three major methods of cytogenetic testing:

1. Routine karyotyping: Karyotyping was one of the first methods of chromosome analysis. This method uses light microscopy and standardized staining procedures on cells in the metaphase portion of the cell cycle, when the chromosomes are lined along the equator of the cell prior to separation and are most condensed.

To make the chromosomal analysis more effective and efficient, stains have been developed to bind with DNA and produce characteristic banding patterns to identify different chromosomes. The stain most commonly used is ‘Giemsa dye’. Through this process, the chromosomes can be organized into a karyogram of 23 pairs and any abnormality involving ‘aneuploidy’(an abnormal amount of chromosomes) and large translocations (where parts of chromosomes are transplanted between each other) can be identified.

Karyotyping can only identify changes over approximately 3 megabases in size. Any abnormality involving less than this will not be picked up by ‘routine karyotyping’. Karyotyping can be used to identify ‘Down syndrome’ and ‘Turner syndrome’.

1. Fluorescent in situ hybridization (FISH): This has rapidly become a well-known diagnostic cytogenetic test in both congenital and acquired disease. FISH has a much higher resolution than routine karyotyping, especially when used on interphase cells (the phase cells remain in when not in mitosis).

FISH uses fluorescent probes with complementary base sequences to locate the presence or absence of specific portions of DNA on chromosomes. The probe and target DNA must be denatured with heat or chemicals to break hydrogen bonds in the DNA and to allow hybridization to occur once the two samples are mixed. The fluorescent probes form new hydrogen bonds with their complementary base pairs on the DNA, and these can then be detected through microscopy.

FISH is commonly used to detect specific chromosomal deletions or translocations associated with pediatric conditions or cancers. Examples include the deletion on chromosome 22 in DiGeorge syndrome and the translocation of part of a gene on chromosomes 22 and 9 in chronic myeloid leukaemia.

FISH is also used for melanocytic lesions to distinguish atypical melanocytic naevi (eg, Spitz naevus) from malignant melanoma.

1. Comparative genomic hybridization (CGH) and array comparative genomic hybridization (aCGH): 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 megabases.

aCGH 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 aCGH 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. **HISTOPATHOLOGICAL TECHNIQUES**: Histopathological examination studies tissues under the microscope. During this study, the pathologist looks for abnormal structures in the tissue.

Tissues for histopathological examination are obtained by biopsy. Biopsy is a tissue sample from a living person to identify the disease. Biopsy can be either incisional or excisional.

Once the tissue is removed from the patient, it has to be immediately fixed by putting it into adequate amount of 10% Formaldehyde (10% formalin) before sending it to the pathologist.

The purpose of fixation is:

1. To prevent autolysis and bacterial decomposition and putrefaction.
2. To coagulate the tissue to prevent loss of easily diffusible substances.
3. To fortify the tissue against the deleterious effects of the various stages in the preparation of sections and tissue processing.
4. To leave the tissues in a condition which facilitates differential staining with dyes and other reagents.

 Once the tissue arrives at the pathology department, the pathologist will exam it macroscopically (i.e. naked-eye examination of tissues).

Then the tissue is processed to make it ready for microscopic examination. The whole purpose of the tissue processing is to prepare a very thin tissue (i.e. five to seven μm or one cell thick tissue) which can be clearly seen under the microscope. The tissue is processed by putting it into different chemicals. It is then impregnated (embedded) in paraffin, sectioned (cut) into thin slices, & is finally stained. The stains can be Hematoxylin/Eosin stain or special stains such as PAS, Immunohistochemistry, and so on.

The Hematoxylin/Eosin stain is usually abbreviated as H&E stain. The H&E stain is routinely used. It gives the nucleus a blue color & the cytoplasm & the extracellular matrix a pinkish color. Then the pathologist will look for abnormal structures in the tissue. And based on this abnormal morphology he/she will make the diagnosis. Histopathology is usually the gold standard for pathologic diagnosis.

1. MICROBIOLOGICAL EXAMINATION: This is a method by which body fluids, excised tissue, etc. are examined by microscopical, cultural and serological techniques to identify micro-organisms responsible for many diseases. Microbiological examination plays an important role in the diagnosis and control of infectious disease. As such examination identifies micro-organisms likely to be involved in the disease and their susceptibility to chemotherapeutic agents, case-specific antimicrobial regimens can be made based on the results. However, results obtained with improper collection, poor technique, inappropriate transport and inadequate management of the specimen may contribute to misdiagnosis and inappropriate antimicrobial therapy.

CELLULAR ADAPTATION PRECEDES CELL DEATH.

The diagram below explains this sequence. Cellular adaptation is the ability of cells respond to various types of stimuli and adverse environmental changes. These adaptations include;

1. Hypertrophy (enlargement of individual cells).
2. Hyperplasia (increase in the number of cells).
3. Atrophy (reduction in the number and size of cells).
4. Metaplasia (transformation of one epithelium to another).
5. 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, fibrinoid, fat, gangrenous and caseous necrosis.

