DEPARTMENT OF PARAMEDICAL SCIENCES

Report of training at Medinova Diagonostic and Clinical Laboratory J&K

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CERTIFICATE

This is to certify that Bakhtawar Hussain Sheikh a student of BSc. MLT from Lovely Professional University bearing Registration no.11013366 has done his semester training at Medinova diagonostic and clinical laboratory Jammu And Kashmir. This is to certify that he has worked under our direct supervision during the training period. His code of conduct during the said period was excellent we wish her very best for the future

(Dr.Pranay Punj Pankaj)

Supervisor

FACULTY OF APPLIED MEDICAL SCIENCES

Acknowledgement

I wish to express my heartful gratitude to my HOD of Pathology, Biochemistry, and Microbiology for their invaluable suggestions, help and guidance. Their enthusiasm, meticulous approach and active interest in work made my task simple and interesting. Their constant guidance and lucid understanding of the subject was largely responsible for the successful completion of this study. I consider myself extremely privileged to have worked under their supervision.

I would also thank my Internal Guide Dr. Pranay Punj Pankaj, Professor, Lovely Professional University, Punjab. I also express my thankfulness to , Mr Javaid Ahmed senior lab technician of Medinova for his constant moral support throughout my training.

My sincere thanks to all technical staff of laboratory of Medinova diagonostic and clinical laboratory J&K

I would be ungrateful on my behalf if I do not thank my family for their unstinted support and constant encouragement.

Place: LPU, Punjab Bakhtawar Hussain Sheikh

Date: 29/04/2015

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ABSTRACT

The project report was based on my 4 months Internship that I had completed from Medinova Diagonostic and Clinical Laboratory and my training period was started from 01-01-2015.From these 4 months I learn a practical knowledge in different departments from this hospital and my duty was in different sections i.e.:-Pathology, Biochemistry, Pathology. The purpose of my project report is to investigate that what number of abnormal results was daily seen in Hospital from different departments and in which condition they come and this helps for patient treatment because treatment was depend on the result. Different tests we perform in different departments. PATHOLOGY Section-Histopathology-Grossing, processing, staining, microscopy and another section was Hematology in we perform CBC, Urine and Coagulation Profile. In Biochemistry section routinely test which we done are blood sugar, liver function test, kidney function test, and lipid profile. In Microbiology department we did different tests in different sections such as Bacteriology, Collection, Media Room, Serology and Immunology.

SECTION – 1

HISTOPATHOLOGY

Histopathology, the study of tissues affected by disease, can be very useful in making a diagnosis and in determining the severity and progression of a disease. Understanding the normal structure and function of different tissues is essential for interpreting the changes that occur during disease. This unit introduces the basic principles that apply to the preparation of microscope sections. It also shows how to identify a number of human tissues and interpret the changes that occur in disease. TheHistopathological specimen are mostly collected by a surgeon in an operation theatre.The specimen in the form of small pieces of tissues are submitted to the histopathology section of the pathology laboratory. Each specimen is immediately placed in a proper fixative and then it is entered in a log book and given unique identification number.

PROCESSING OF TISSUE SPECIMEN IN HISTOPATHOLOGY SECTION

RECEPTION

On arrival in the departmental reception, the specimen is checked at the earliest opportunity for the following:

1. That the specimen is for histological examination.

2. That the container is clearly labeled and accompanied by a completed request form.

3. That sufficient fixative is in the container, or if the specimen is not in fixative or is in a wrong fluid. The request form is dated and stamped; the specimen is given as identification serial number which remains with the specimen until all the investigations have been carried out.

GROSSING: (Only observing)

It is done by Pathologist Take only few amount of infected tissue like lobes bone, infected cyst etc. in the cascade for examination after processing and staining.

Tissues removed from the body for diagnosis arrive in the Pathology Department and are examined by a pathologist, pathology assistant, or pathology resident. Gross examination consists of describing the specimen and placing all or parts of it into a small plastic cassette which holds the tissue while it is being processed to a paraffin block. Initially, the cassettes are placed into a fixative.

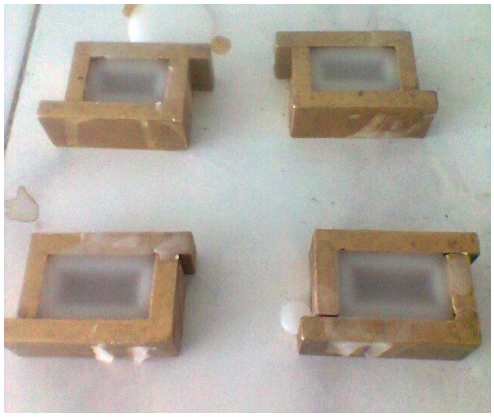
Tissue Processing:

Processing of tissue was done by automated tissue processor (histokinette) for 12 hours.

- 1O% Formalin – 1 hour.
- 1O% Formalin – 1 hour.
- 70% Ethyl Alcohol – 1 hour.
- 80% Ethyl Alcohol – 1 hour.
- 95% Ethyl Alcohol – 1 hour.
- 95% Ethyl Alcohol – 1 hour.
- Absolute Alcohol – 1 hour.
- Absolute Alcohol – 1 hour.
- Xylene – 1 hour.
- Xylene – 1 hour.
- Wax – 1 hour.
- Wax – 1 hour.

Embedding

After processing the specimens are placed in an embedding center where they are removed from their cassettes and placed in wax-filled molds. At this stage specimens are carefully orientated because this will determine the plane through which the section will be cut and ultimately may decide whether an abnormal area will be visible under the microscope. The cassette in which the tissue has been processed carries the specimen identification details and it is now placed on top of the mold and is attached by adding further wax. The specimen "block" is now allowed to solidify on a cold surface and when set the mold is removed. The cassette, now filled with wax and forming part of the block, provides a stable base for clamping in the microtome. The block containing the specimen is now ready for section cutting.



Sectioning:

Sections are cut on a precision instrument called a “microtome” using extremely fine steel blades. Paraffin sections are usually cut at a thickness of 3 - 5µm ensuring that only a single layer of cells makes up the section. One of the advantages of paraffin wax as an embedding agent is that as sections are cut they will stick together edge-to-edge, forming a “ribbon” of sections. This makes handling easier. Sections are now “floated out” on the surface of warm water in a flotation bath to flatten them and then picked up onto microscope slides. After thorough drying they are ready for staining.

Staining

The embedding process must be reversed in order to get the paraffin wax out of the tissue and allow water soluble dyes to penetrate the sections. Therefore, before any staining can be done, the slides are "deparaffinized" by running them through xylenes (or substitutes) to alcohols to water. There are no stains that can be done on tissues containing paraffin. The staining process makes use of a variety of dyes that have been chosen for their ability to stain various cellular components of tissue. The routine stain is that of hematoxylin and eosin (H and E).

CASE: 1

Patient Name: Kaushalya

Mrd. No: 1300069107

Age/Gender: 40/Female

Specimen: Cervix Biopsy

H&E Staining was performed.

Hematoxylin& Eosin Principle:-

Hematoxylin and eosin are the principle stain used for the demonstration of nucleus and cytoplasmic inclusion.

Alum acts as a mordent &hematoxylene containing an alum stain the nucleus light blue when turn red in the present of acid tissue with acid solution when imparts pink color of cytoplasm.

Hematoxylin Stain process:-

1. After cutting section dewax with xylene for 5 mins.
2. Dip in xylene II for 5 mins.
3. Dip slide in90% ethanol for 2 min.
4. Then in 80%ethanol for 2 min.
5. Then in 70%ethanol for 2 min.

6. Wash in running tape water for 2 min.
7. Dip in Mayer's hematoxylin for 3 min.
8. Wash with running tape water for 2 min.
9. Stain in 1 % eosin for 1minutes.
10. Wash with running tap water.
11. Dehydrate with 50%, 70%, 90% ethanol.
12. Air dry and mount with mounting media DPX...
13. Examination under microscope.

Result: Nucleus – Blue.

Cytoplasm – Pink.

CYTOLOGY

The cytology laboratory is geared to the study of single cells mainly in detecting malignant and premalignant conditions as well as identifying infectious agents. The laboratory maintains ongoing proficiency testing and performance improvement programs.

The following specimens are examined:

Exfoliative cytology.

a. Gynecologic cervico/vaginal smears
b. Non-Gynecologic specimens

i. Sputum

ii. Bronchial washings brushings and lavages

iii. Body cavity effusions

iv. Nipple discharge

v. Cerebrospinal fluid

vi. Urine

vii. Endoscopic brushings and washings

viii. Other

B. **FINE NEEDLE ASPIRATIONS (FNA):**

a. FNAC is very valuable for preliminary diagnosis of carcinomas as well as inflammatory conditions. Palpable masses performed by the pathologist: These include any palpable mass such as breast, lymph node, thyroid, soft tissue, etc.

Case 2

Patient Name: Indu Sheema

Mrd. No. 11177761

Age/Gender: 32/Female

Ref. Doctor: Dr. Disha

Investigation: Cytological exam. OfPAP smear

PAP Staining was performed

PRINCIPLE OF PAPANICOLAOU STAIN

The most widely used staining procedure for cytological specimens is Papanicolaou's technique. In the first staining step the nuclei are stained by a haematoxylin solution. Nuclei are stained blue, dark violet to black. The second staining step is cytoplasmic

staining by orange staining solution, especially for demonstration of mature and keratinized cells. The target structures are stained orange in different intensities. In the third staining step the so-called polychromatic solution is used, a mixture of eosin, light green SF and Bismarck brown. The polychromatic solution is used for demonstration of differentiation of squamous cells e.g. cervical cancer and cycle diagnosis for examination under microscope.

PAP Staining Procedure:-

1. Prepare the smear and air dry it.
2. Fix the smear in alcohol for 15 min.
3. Rinse in water for 5 mins
4. Hematoxylin for 2 min.
5. Wash with tap water for 5 mins.
6. Rehydrate in 95% alcohol for 2 mins.
7. Again, Rehydrate in 95% alcohol for 2 mins.
8. Stain with OG-6 2mins.
9. Dip in 95% alcohol 2mins.
10. Again dip in 95% alcohol 2mins.
11. Counter stain with EA36 2 min.
12. Rinse in 95% alcohol for 2 mins.
13. Rinse in 95% alcohol for 2 mins.
14. Take the xylene dip.
15. Mounting with DPX.
16. Examine under microscope.

RESULT:

Cytoplasm Cyanophilic (basophilic)- Blue-green.

Cytoplasm Eosinophilic (acidophilic)– Pink.

Cytoplasm Keratinized - Pink-orange.

Erythrocytes - Red.

Nuclei Blue - dark violet, black.

Microorganisms - Grey-blue.

Trichomonas - Grey-green.

PRINCIPLE OF MAY-GRUNWALDS GIEMSA STAIN:

May-Grunwaldsstains acidophilic cells and the neutrophilicgranulations of leukocytes, whereasGiemsa stains the cytoplasm of monocytes and lymphocytes, as well as nuclear chromatin.

Procedure: We first pre-label the slide and spread the collected sample evenly. Fix the slide in the fixative (Methanol) for 15 minutes. Stain the slides with MGG staining. It is the type of Romanowsky Stains. It is also called as Azure that means it is made of more than two stains. It contains acidic and basic dye. Acidic part takes basic dye and basic part takes acidic dye. After fixing of slides we pour off slides with 1:1 ratio of May Grunewald's stain for 8 minutes.After that drain the stain from the slides by first washing.Pour second stain i.e., Giemsa 1:9 ratio on the slides for 12 minutes. Blot the slides and mount with DPX.

Result: Nucleus – Purple.

Cytoplasm– Blue.

Red Blood Cells – Pink

SECTION

HAEMATOLOGY

Hematology is the study of blood and is concerned primarily with the study of the formed elements of the blood.These include erythrocytes (RBC), leucocytes (WBC), andThrombocytes (PLT).The hematology laboratory routinely reports the enumeration of cells in circulation, hemoglobin concentration and differential count of leukocytes based on the study of the stained blood smear. Study of the blood smear also helps in detecting the morphological abnormalities of various cells seen in the peripheral blood circulation.Another aspect of the hematology laboratory is to investigate causes of bleeding disorders.

Sections of Hematology:

- Reception.
- CBC Analyzer.
- Coagulogram Analyzer.
- Urine Processing.
- Reporting.

SYSMEX XS 800i FULLY AUTOMATED HAEMATOLOGY ANALYZER

Introduction

The Sysmex XS-800i is a compact new, fully automated haematologyanalyser, designed to generate complete blood counts with five-part leucocyte differential. A Sysmex XS-800i instrument was evaluated according to Clinical Laboratory Standards Institute (CLSI) and International Council for Standardization in Haematology (ICSH) guidelines. Precision, carry-over and linearity were determined. Using quality control material, total

and within-run imprecision was less than 3% except for platelets. The system demonstrated good linearity over the entire reporting range and no carry-over (<0.5%). Overall flagging sensitivity and specificity were 91% and 48%, respectively. In conclusion, the Sysmex XS-800i demonstrated good analytical performance, is able to generate a complete blood count with five-part differential on low blood volumes and has considerable back-up capacity.

Principle of CBC counter:-

Sysmax developed Electric Capacitance method rathor then the resistance
Method when the partical is located in the detection area a change in electrical capacitance occurs and this proportional to the volume of the partical, it is possible the count the total no. of cells as they pass through the detection area.

In another word, when the diluted blood coming in the contact with the Capacitance, the electrical current is absorbed by blood cells, the amount of current is Absorbed by cells detector is detect the current and count the cells.

Samples on Automatic rotator

Process of this test:-

- When the sample coming from collection center.
- Rotate the sample on Automatic rotator

For Sysmex 800i counter

- Machine should be on before running the sample
- press Sample no. (Enter the file no. or request no.)
- Then press enter key.
- Aspirate the sample in aspiration areas
- Wait for few minute and print will ready.

Case No: 1

Age: 63 Yrs /male

Mrd No: 12008395

Sample Date: 07/01/2015
Report Date: 10/001/2015
Doctor Name:Dr. Mukhtar

Complete blood count

Investigation	Results	Ref.Range/Unit
• Haemoglobin	5.7	11.0-16.0 g/dl
• Haematocrit	22.0	33-48 %
• RBC Count	2.36	2.5-5.5 million/cumm
• MCV	93.2	80-96 fl
• MCH	24.1	27-38 pg
•MCHC	25.931-37 g/dl	
• TLC	2,500	4000-11,000 Cells/cumm
• DLC		
• Neutrophils	41	37-72 %

• Lymphocyte	38	25-48 %
• Monocyte	03	0-11 %
• Eosinophils	08	00-06 %
• Basophils	00	00-01%
• Platelets count	1, 20,000	1, 50,000 - 4, 00,000

Clinical significance: -A decrease in Hb concentration in blood below normal values is a sign of anemia. The Hb concentration is lower in adult women as compared to adult males.Hb values further drops during pregnancy due to haemodilution.

Case No- 2

Age: 28 Years / female

Mrd No: 130003779

Sample Date: 12/01/2015

Report Date: 14/01/2015

Doctor Name: Dr. Fatima

Complete blood count

Investigation	**Results**	**Ref.Range/Unit**
• Haemoglobin	12.9	11.0-16.0 g/dl
• Haematocrit	42.1	33-48 %
• RBC Count	3.39	2.5-5.5 million/cumm
• MCV	111.1	80-96 fl
• MCH	38.05	27-38 pg
•MCHC	30.6	31-37 g/dl
• TLC	12,520	4000-11,000 Cells/chum

• DLC		
• Neutrophils	70	37-72 %
• Lymphocyte	17	25-48 %
• Monocyte	01	0-11 %
• Eosinophils	06	00-06 %
• Besophils	00	00-01%
• Platelets count	2, 26,000	1, 50,000 - 4, 00,000

Clinical significance:-Increased MCV is a sign of macrocytosis, which may be related to anemia due to deficiency of vitamin B12 and Folic acid with the occurrence of megloblastic anemia.

E.S.R

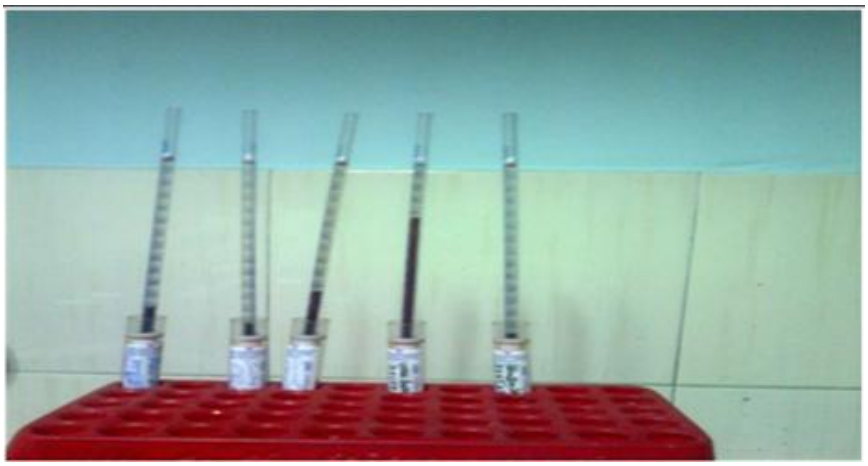
Requirements: ESR tubes, Rack, Timer, and Anticoagulated blood (Sodium Citrate).

Procedure:

- Remove the cap on the prefilled anticoagulated vial of blood.
- Insert the pipette through the mouth of the tube it will aspirate blood upto specific level.
- Let the pipette stand for one hour, then read the numerical results of the ESR.
- Dispose of all bio hazardous waste in the appropriate containers.

Principle:-

The speed at which the red blood cells in normal blood settle is relatively slow. However in many diseases, change occurs in physiological properties of plasma. Change in the surface electrical charge of the red blood cells caused the erythrocyte to aggregate clump, or to form rouleaux .The large clump of cells thus forms fall at a fast rate. The changes in the proportion of the soluble constituents of plasma such as increased fibrinogen of globulin also result in increased rateof erythrocyte fall.



Case: 1

Age: 30 Years / Male

Sample Date : 16/01/2015
Mrd. No.:130002835

Investigation	Results	Ref.Range/Unit
• Erythrocyte sedimentation rate	130 mm/hr	0 - 20 mm/hr

Case: 2

Age:23 Years / Male

Sample Date : 20/01/2015

Mrd. No.:130005383

Investigation	Results	Ref.Range/Unit
• Erythrocyte sedimentation rate	10 mm/hr	0 - 20 mm/hr

Interpretations:

ESR is increased in all conditions where there is tissue breakdown or where there is entry of foreign proteins in the blood, except for localized mild infections. The determination is useful to check the progress of the disease. If the patient is improving the ESR tends to fall. If the patient's condition is getting worse the ESR tends to rise. The changes of the ESR are, however, not diagnostic of any specific disease.

Urine routine & Microscopic

Routine urine analysis is mainly performed for two purposes:-

- To find out metabolic or endocrine distribution of the body.
- To detect intrinsic conditions that may adversely affect the urinary tract or kidney.

Procedure:-

1. Take about 5 ml of urine in a RIA vial.
2. Liable properly and centrifuge them.
3. Discard the supernatant .
4. Place the one drop of sediment on the glass slide and place the coverslip
5. Examine under microscope at 40x

Principle: -The microscopic elements present in urine (in suspension) are collected in the form of deposit by centrifugation. Small drop of the sediment is examined by making a coverslip preparation under microscope.

Case: 1

Mrd. No: 130002249

Ref. Doctor: Dr.Muzafar

Date: 26/01/2015

Physical Examination

- Total volume 40ml
- Color Pale Yellow
- Turbidity Clear
- Reaction Acidic

Clinical Examination:-

- Albumin Nil
- Sugar Nil

Microscopic Examination:-

- Pus Cells 40 - 50 /hpf
- RBCs Nil / hpf
- Epithelial cells 2 - 4 / HPF
- Cast Nil / HPF
- Crystals Nil / hpf
- Significance cells Nil.

Case 2

Mrd. No: 130001093

Ref. Doctor: Dr. Muzafar

Date: 28-01-15

Physical Examination

- Total volume
- Color Pale Yellow
- Turbidity Clear
- Reaction Acidic

Clinical Examination:-

- Albumin Nil
- Sugar Nil

Microscopic Examination:-

- Pus Cells 40 - 50 /HPF
- RBCs Nil / HPF
- Epithelial cells 2 - 4 / HPF
- Cast Nil / hpf
- Crystals Nil / hpf

Interpretations:

Protein-Nephrotic syndrome, Pyelonephritis, Glomerulonephritis, Malignant hypertension.

Glucose: Lowered renal threshold, Renal tubular disease, Diabetes Mellitus, Pancreatitis.

Pus: An increase number of WBC's in the urine indicates renal infection, which can be in the bladder (cystitis) or in the kidney (pyelonephritis).

Epithelial Cell: Presence of few squamous cells (from urethra) is considered to be normal. Dirty appearing tubular cells are seen in acute tubular necrosis. Presence of transitional cells (from bladder or ureters) might suggest transitional cell carcinoma. [9]

Red Blood Cell: Haemoglobinuria might result from haemolytic anemia, incompatible blood transfusion or paroxysmal haemoglobinuria(PNH), whilehaematuria might indicate bleeding in the urinary tract.

COAGLOGRAM PROFILE

In Coagulation Lab the following investigations were performed, Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Bleeding time (BT) and Clotting time (CT).

Procedure:

- Pipette 50 µL plasma into a reaction tube and set it into detector by pushing it into bottom of the detector firmly
- Press the start button. Incubation of plasma will start and the countdown of 180 seconds will begin, the detector LED blinks red.
- When the time of reagent addition comes, an alarm will begin to sound. The detector LED will blink green.
- When alarm begins to sound take the reagent 100 µL and add with a pipette without forming bubbles inside the wall of reaction tube and close the lid.
- When the reagent is added, mixing will start automatically and detector LED will light up in red.
- In case of APTT 2nd reagent is required the countdown will begin again and 2nd reagent is added and the result is read out on the display.

Observations:

PTI	Name	Age/Sex	MRD NO.	Result	Normal Range
Case 1	Showkat	38/M	130002654	39.0	11.5 – 15.5

Case 2	Shahnawaz	36/M	130002091	17.5	11.5 – 15.5

INR	Name	Age/Sex	MRD NO.	Result	Normal Range
Case 1	Showkat	38/M	130002654	2.6	0.8 – 1.2
Case 2	Shahnawaz	36/M	130002091	1.1	0.8 – 1.2

Interpretations: PT may be prolonged due to lack of vitamin k absorption in obstruction or lack of synthesis in hepatocellular disease. The PT is abnormal in coagulation defects due to liver disease because it is affected by deficiencies of more than one factor.PT is normal or slightly prolonged in majority of patients with acute infectious or toxic hepatitis and also in chronic liver disease.

CA-50 BLOOD COAGULATION ANALYZER

The CA-50 is an automated blood coagulation analyzer that can quickly analyze sample with a high degree of accuracy. The CA-50 can analyze sample using a coagulation method and the analyzed data can be displayed on its LCD screen and printed by the built in printer. The CA-50 also has some supplemental functions including quality control.

Analysis Parameters and calculation Parameters: The following list shows the analysis and calculation parameters that can be analyzed by the CA-50

Method	Analysis Parameters
Coagulated method	PT (Prothrombin Time),
	APTT (ActivatedPartialThromboplastin Time),
	Fibrinogen, ThrombinTime (TT), Normotest,

Analysis Principle of CA-50:

Coagulation reaction detection method (scattered light detection method) irradiates red light (660nm) onto a mixture of blood plasma and reagent and detects the change in turbidity (when the fibrin clots are formed) as the change in scattered light and measures the coagulation time.

Blood grouping

There are nearly 300 blood group systems so far discovered. The ABO and Rh are the major,clinically significant and the most important of all the blood group systems.All people (with few exceptions) of ABO system can be divided into 4 major groups in this system and they are agroup, Bgroup, AB group,O group.This depends on the reactions obtained by mixing their red blood cells to different reagents,known as Anti-A,Anti-B.

Slide method: - Take three drop of blood sample on a slide and place the monoclonal antibody each drop of blood sample.

Observe the agglutination of blood.

If agglutination occur in A sera Blood group is- "A"

If agglutination occur in B sera Blood group is- "B"

If agglutination occur in AB sera Blood group is- "AB"

If no agglutination occur in AB sera Blood group is- "O"

ABO Grouping:-

Reaction	Monoclonal Antibodies A	Monoclonal antibodies B	Monoclonal antibodies D	Result
Agglutination	+	-	+	A positive
Agglutination	+	-	-	A Negative
Agglutination	-	+	+	B Positive
Agglutination	-	+	-	B Negative
Agglutination	+	+	+	AB Positive
Agglutination	+	+	-	AB Negative
Agglutination	-	-	+	O Positive
Agglutination	-	-	-	O Negative

Keys

- + =Agglutination.
- -=No agglutination

SECTION – 2

BIOCHEMISTRY

Clinical Biochemistry deals with the biochemistry laboratory applications to find out cause of a disease. The chemical constitutes of various body fluids such as blood(serum or plasma),urine,CSF and other body fluids are analyzed in clinical biochemistry laboratory. These determinations are useful in diagnosing various clinical conditions such as diabetes mellitus,jaundice,gout,hyperlipidemia,pancreatitis, rickets etc.The biochemistry tests are very useful in determining the severity of diseases of many organs such as liver,stomach,heart,kidneys,brain as well as endocrine disorders and related status of acid-base balance of the body. The clinical biochemistry tests, in relation to the various clinical conditions can:-

- Reveal the cause of the disease.
- Screen easy diagnosis.
- Suggest effective treatment.
- Assist in monitoring progress of pathological conditions and
- Help in assessing response to therapy.

Collection and separation of samples: In biochemistry we received the samples from OPD/IPD for analyses in vacutainerhaving red top/cap that indicated the samples are without anticoagulant. But for some specific test such as HbA1c samples were collected in purple top/cap tubes. The tubes werecentrifuged at 3000 RPM.

The most important automated analyzer in the laboratory was Mindray BS 400, the laboratory also possessed EM 360, and it also hadErbaChem 7 a semi-automated analyzer for emergency parameters.

These are the test which is performed in BS400 Analyzer in the laboratory and there procedure is same.

1. Glucose

- Blood sugar Random (BSR)
- Blood sugar Fasting (BSF)
- Blood Sugar Post Prandial (BSPP)
- HbA1c

2. Kidney function test (K.F.T.)

- Urea
- Serum creatinine.
- Blood Urea Nitrogen(BUN)

3. Liver Function Test (L.F.T)

- Bilirubin Direct and Total
- SGPT,
- SGOT,
- ALP,

4. Lipid Profile.

- Cholesterol
- Triglyceride
- HDL

5. Uric Acid.

Principle and working of BS400:-

The Principle of BS400 is based on the Spectro photometer, is based on the Beer's Law. The amount of light absorbed by the colouredsolution, when illuminated with light of suitable wavelength is directly proportional to the concentration of the colour solution and length of the light path thought the solution therefore the amount of light decrease exponentially with increase the concentration of the solution and which increase the thickness of the layer of solution thought which light passes or Which state that optical density is directly proportional to the concentration of colouredsolution.

Procedure to run the sample on Mindray BS 400

- Check all the cans of distilled water, cleaning solution and water.
- Switch on the UPS and then the computer.
- Switch on the analyzer, water filter and instrument refrigerator.
- Wait for system initialization.
- Click on Calibration
- Select the Parameters
- Click on QC
- Select the test and request
- Keep saline at S1, Control (Normal and Pathological) C1, C2 and calibrator at S2.
- Click on run test.
- Click on sample and put patient ID
- Select the test and request
- Click on run test
- The analyses will begin and the results will be displayed on the screen.

Case 1

Blood Glucose

Principle(Glucose oxidase Peroxidase): Glucose oxidase catalyses the oxidation of glucose to produce hydrogen peroxide and gluconic acid. The hydrogen peroxide, in the presence of enzyme peroxidase is broken down and the oxygen given off reacts with 4- amino antipyrine and phenol to give a pink color

Patient Name: Shafi

Mrd.No:130005844

Age/Sex: 32/F

Investigation: Blood Sugar Random

Result: 195

Normal Range: 90-140 mg/dl.

Clinical Significance: Increased value of Glucose in the case of Diabetic mellitus, Endocrine disorder, acromegaly, stress, chronic renal failure, Pancreatitis, Drugs, steroids, thiazides, oral contraceptives etc.

Decreased values in the case of Insulinoma, Hypopituitarism, Adrenal, Adrenal cortical insufficiency, Sever liver disease, Extra pancreatic neoplasm, Ethanol ingestion, Drugs. Etc.

Case 2

Serum Bilirubin

Principle

Diazo method

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with a diazonium ion in a strongly acidic medium (pH 1-2).

Bilirubin + diazonium ion $\xrightarrow{\text{acid}}$ azobilirubin

The intensity of the color of the azobilirubin produced is proportional to the total bilirubin concentration and can be measured photometrically.

Patient Name: Rafia
Age: 19 Years / Female
Mrd. no: 130005843
Sample Date: 02/02/2015
Doctor Name: Dr.Sheeraz

Result: Total bilirubin: - 4.0mg/dl

Direct bilirubin: - 0.8 mg/dl

Normal Range: Total bilirubin: - 0.3-1.0 mg/dl

Direct bilirubin: - 0.1 -0.4 mg/dl

Indirect bilirubin: - 0.2-0.7 mg/dl

Clinical Significance: Serum total bilirubin is increased in hepatocellular damage (infectious hepatitis, alcoholic and other toxichepatopathy, neoplasms), intra- and

extrahepatic biliary haemolysis, physiologic neonatal jaundice, Crigler-Najjar syndrome, Gilbert's disease, Dubin-Johnsonsyndrome, and fructose intolerance.

Elevation of direct (conjugated) bilirubin is seen in cholestasis and late in the course of chronic liver disease. Indirect (unconjugated) bilirubin tends to predominate in haemolysis and Gilbert's disease. Decreased serum total bilirubin is probably not of clinical significance but has been observed in iron deficiency anemia. [22]

Case No- 3

ALKALINE PHOSPHATASE

Patient Name: Monika
Age: 23 Years /female
Mrd no: 130005248
Sample Date:05/02/2015
Doctor Name: Dr.Mukhtar

Normal Range: Adult: 25-100 U/L

Children: Less than 350 U/L

Result: 284 U/L

Principle

Colorimetric assay in accordance with a standardized method.

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.

P-nitrophenylphosphate +H_2O $\xrightarrow{ALP}$ phosphate + p-nitrophenol

The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

Clinical Significance

- Very high levels of ALP can be caused by liver problems, such as hepatitis, blockage of the bile ducts (obstructive jaundice), gallstones, cirrhosis, liver cancer, or cancer that has spread (metastasized) to the liver from another part of the body.
- High ALP levels can be caused by bone diseases, such as Paget's disease, osteomalacia, rickets, bone tumors, or tumors that have spread from another part of the body to the bone, or by overactive parathyroid glands (hyperparathyroidism). Normal healing of a bone fracture can also raise ALP levels.
- Heart failure, heart attack, mononucleosis, or kidney cancer can raise ALP levels. A serious infection that has spread through the body (sepsis) can also raise ALP levels.

Case No- 4

AST (SGOT)

Patient Name: Abdul Rehman
Age: 45 Years / Male
Mrd. No: 130005108
Sample Date: 10/02/2015
Doctor Name: Dr. Mukhtar

Result: **117** IU/L

Normal Range: 10-40 IU/L

Principle

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD+.

Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

L-Aspartate + 2-oxoglutarate $\xrightarrow{\text{AST}}$ oxaloacetate + L-glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{\text{MDH}}$ L-malate + NAD+

The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

Clinical Significance

An increase in AST levels may indicate:

Acute hemolytic anemia
Acute pancreatitis
Acute renal failure
Liver cirrhosis
Heart attack
Hepatitis
Infectious mononucleosis
Liver cancer
Liver necrosis

Case No- 5

ALT (SGPT)

Patient Name: Bhura Ram

Age: 45 Years / Male

Mrd. No: 130005108

Sample Date: 18/02/2015

Doctor Name: Dr. Muzafar

Result: 145 IU/L

Normal Range: 7-56 IU/L

Principle

AST catalyzes the reaction between L-alanine and 2-oxoglutarate.The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate andNAD^+.

Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

L-Alanine + 2-oxoglutarate $\xrightarrow{\text{ALT}}$ pyruvate + L-glutamate

Pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ L-lactate + NAD^+

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

Clinical Significance

Greater-than-normal ALT levels may indicate:

Celiac disease

Cirrhosis

Hepatitis (viral, autoimmune)

Hereditary hemochromatosis

Liver ischemia (blood flow deficiency to the liver)

Liver tumor

Use of drugs that are poisonous to the liver

Case No- 6

CHOLESTEROL

Patient name – Shingara Ram

Age - 46 /Male

Mrd.no:130005208

Sample date- 04/03/2015

Doctor name- Dr.Fatima

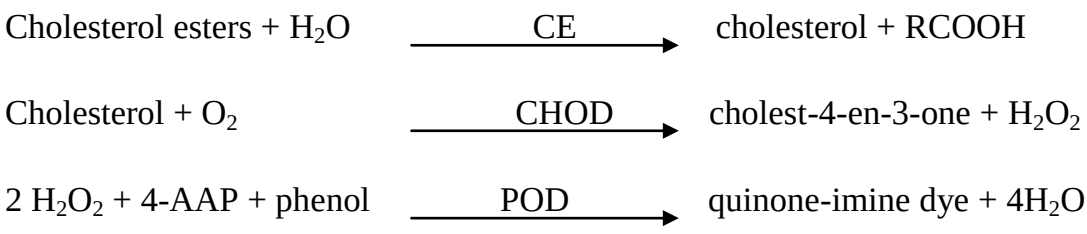
Result: 295 mg/dl

Normal Range: less than 200 mg/dl

Principle

Enzymatic, colorimetric method.

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinine-imine dye.



The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.

Clinical Significance

Increased in: 1) Diabetes mellitus
2) Nephrosis
3) Biliary cirrhosis
4) Lipoprotenemias
5) Hypothyroidism

Decreased in: 1) Severe infection
2) Severe anemia
3) Hyperthyroidism
4) Malnutrition

Case No- 7

URIC ACID

Patient name – Gh. Qadir

Age - 70 /Male

Mrd. No - 130005532

Sample date- 10/03/2015

Doctor name- Dr. Mukhtar

Result: 7.1 mg/dl

Normal Range: Men- 2 to 7.5 mg/dL,

Women- 2 to 6.5 mg/dL.

Principle

Enzymatic colorimetric test.

Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

Uric acid + 2 H_2O + O_2 Uricase allantoin + CO_2 + H_2O_2

$\longrightarrow$

2 H_2O_2+ H^+ + TOOSa + 4-aminophenazone peroxidase quinone-diimine

$\longrightarrow$

dye + 4 H_2O

a) N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

The color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance.

Clinical Significance

Increase in serum uric acid is seen idiopathically and in renal failure, disseminated neoplasms, toxemia of pregnancy, psoriasis, liver disease, ethanol consumption, etc. Many drugs elevate uric acid, including most diuretics, catecholamines, ethambutol, pyrazinamide, salicylates, and large doses of nicotinic acid.

Decreased serum uric acid level may not be of clinical significance. It has been reported in Wilson's disease, Fanconi's syndrome, and Hodgkin's disease, myeloma, and bronchogenic carcinoma.

Case no-8

UREA

Patient name – Gani kak

Age - 55 /Male

Mrd. No - 130005374

Sample date- 14/03/2015

Doctor name- Dr.Humaira

Result: 186 mg/dl

Normal Range: 17-56 mg/dl,

Principle

Kinetic test with urease and glutamate dehydrogenase. Urea is hydrolyzed by urease to form ammonium and carbonate.

$$Urea + 2H_2O \xrightarrow{Urease} 2\,NH_4^+ + CO_3^{2-}$$

In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD for each mole of urea hydrolyzed.

NH_4^+ + 2-oxoglutarate + NADH $\xrightarrow{\text{GLDH}}$ L-glutamate + NAD^+ + H_2O

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically.

Clinical Significance

An abnormally high level of urea nitrogen in the blood is an indication of kidney function impairment or failure. Some other causes of increased values for urea nitrogen include prerenal azotemia (e.g. shock), postrenal azotemia, GI bleeding and a high protein diet. Some causes of decreased values for urea nitrogen include pregnancy, severe liver insufficiency, overhydration and malnutrition.

Normal range: 17-56 mg/dl.

Result: 120 mg/dl

Case No- 9

CREATININE

Patient name – Mohd. Jabbar
Age - 55 /Male
Mrd. No - 130005374
Sample date- 21/03/2015
Doctor name- Dr. Sheeraz

Result: 5.1 mg/dl

Normal Range: 0.6-1.4mg/dl,

Principle

This kinetic colorimetric assay is based on the Jaffe method. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen.

Creatinine + picric acid $\xrightarrow{\text{Alkaline pH}}$ yellow-red complex.

Clinical Significance

Creatinine is a breakdown product of Creatine. Measurement of creatinine levels is used as one indicator of kidney function. Serum creatinine is a waste product of the dehydration of Creatine. Most of the body Creatine is present in muscle tissue. It represents an important alternative energy source for the body. The primary source of energy for the body is the conversion of ATP to ADP by breaking a high-energy phosphate bond and releasing energy that can be used. Creatine is important as it is an efficient energy source used to covert ADP back to ATP.

Case 10: HbA1c

Principle: Glycosylated Hemoglobin (GHb) has been defined operationally as the fast fraction hemoglobinsHbA1 (HbA1a, A1b, A1C) which elute first during column chromatography. The non-glycosylated hemoglobin, which consists of the bulk of haemoglobin, has been designated HbAo.

A hemolysed preparation of whole blood is mixed continuously for 5 minutes with a weakly binding cation-exchange resin. The labile fraction is eliminated during the hemolysate preparation and during the binding. During the mixing, HbAo binds to the ion exchange resin leaving GHb free in supernatant. After the mixing period, a filter separator is used to remove the resin from the supernatant. The percent glycosylated hemoglobin is determined by measuring absorbance of the glycosylated (GHb) fraction and the total (THb) fraction. The ratio of the Glycosylated hemoglobin and the Total hemoglobin fraction of the control and the test are used to calculate the percent Glycosylated hemoglobin of the sample. Requirements:

Reagent-Ion Exchange Resin (Predispensed Tubes), Lysing reagent, Control (10% GHb), Resin separators. Blood sample (EDTA) Reagent Preparation: The Ion Exchange resin tubes and the lysing reagent are ready to use. Reconstitute the control with 1ml of distilled water. Allow to stand for 10 minutes with occasional mixing. The reconstitute control is stable for at least 7 days when stored at 2-8^{o}c.

Wavelength: 415 nm

Procedure:**A)**Hemolysate preparation.

1. Dispense 0.5 ml lysing reagent into tubes labelled as Control(C) & Test.

2. Add 0.1 ml of the reconstitute control & well mixed blood sample into the appropriately labelled tubes. Mix until complete lysis is evident.

3. Allow to stand for 5 minutes.

B) Glycosylated hemoglobin (GHb) separation

1. Remove cap from the Ion exchange resin tubes and label as Control and Test.

2. Add 0.1 ml of the hemolysate from step A into the appropriately labelled Ion exchange resin tubes.
3. Insert a resin separator into each tube so that the rubber sleeve is appropriately 1cm above the liquid level of the resin suspension.
4. Mix the tubes on a rocker continuously for 5 minutes.
5. Allow the resin to settle, and then push the resin separator into the tubes until the resin is firmly packed.
6. Pour or aspirate each supernatant directly into a cuvette and measure each absorbance against distilled water.

C) Total Hemoglobin (THb) fraction

1. Dispense 5.0 ml of distilled water into tubes labeled as Control and Test
2. Add to it 0.02 ml of hemolysate from step A into appropriately labeled tube. Mix well
3. Read each absorbance against distilled water

Calculations: Ratio of Control (R_c) = Abs. Control GHb/Abs. Control THb

Ratio of Test (R_t) = Abs Test GHb/Abs Test THb

GHb in % = Ratio of test (R_t)/Ratio of Control (R_c) ×10(Value of Control)

Case 1

Patients
Name:Ravinder
Singh.
Age/Sex: 22/Male
MRD No.
120078890
HbA1c 8.40

Case 2

Patients Name:

Ravinder Singh.

Age/Sex: 22/Male

MRD No.120078890

HbA1c 9.40

Mean Blood Glucose (MBG) in mg/dl = 33.3×HbA1c Value – 86.These values are linear in the range of 6.5 – 13% of HbA1c values.

Normal reference values:

Normal:< 8.0%

Good Control: 8.0 – 9.0%

Fair Control: 9.0 – 10.0%

Poor Control:> 10.0%

Clinical significance: GHb reflects the metabolic control of glucose level over a period of time unaffected by diet, insulin, other drugs or exercise on the day of testing. GHb is now widely recognized as an important test for the diagnosis of Diabetes Mellitus and is a reliable indicator of the efficacy of therapy.

SECTION 3

MICROBIOLOGY

Introduction: Microbiology involves the study of microscopic organisms. Although microorganisms are generally beneficial and essential for life, some are, however, pathogenic and cause infectious diseases. The diagnostic microbiology laboratory is engaged in the identification of infectious agents. These infectious agents are broadly classified as viruses, bacteria, mycotic agents and parasites (protozoa and helminths).Identification of the infectious agent is the principle function of diagnostic microbiology laboratory. In addition, the laboratory also provides guidance in therapeutic management. This is particularly true in the case of bacterial infection where the laboratory provides information regarding the most effective antimicrobial agent and its dosage to be used for the specific patient.

Sections of Microbiology Laboratory:

- Media Preparation
- Collection
- Bacteriology
- Serology
- Immunology

Bacterial culture media:

One of the most important reasons for culturing bacteria in vitro is its utility in diagnosing infectious diseases. Isolating a bacterium from sites in body normally known to be sterile is anindication of its role in the disease process. Culturing bacteria is also the initial step in studying its morphology and its identification. Bacteria have to be cultured in order to obtain antigens from developing serological assays or vaccines. Certain genetic studies and manipulations of the cells also need that bacteria be cultured in vitro. Culturing bacteria also provide a reliable way estimating their numbers (viable count). Culturing on solid media is another convenient way ofseparating bacteria in mixtures.

History:

Louis Pasteur used simple broths made up of urine or meat extracts. Robert Koch realized the importance of solid media and used potato pieces to grow bacteria. It was on the suggestion of Fannie Eilshemius, wife of Walther Hesse (who was an assistant to Robert Koch) that agar wasused to solidify culture media. Before the use of agar, attempts were made to use gelatin assolidifying agent. Gelatin had some inherent problems; it existed as liquid at normal incubating temperatures (35-37oC) and was digested by certain bacteria.

Composition of culture media:

Bacteria infecting humans (commensals or pathogens) are chemoorganoheterotrophs. When culturing bacteria, it is very important to provide similar environmental and nutritional conditions that exist in its natural habitat. Hence, an artificial culture medium must provide all the nutritional components that a bacterium gets in its natural habitat. Most often, a culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors. Besides these, the pH of the medium must be set accordingly. Some of the ingredients of culture media include water, agar, peptone, casein hydrolysate, meat extract, yeast extract and malt extract.

Classification:

Bacterial culture media can be classified in at least three ways; Based on consistency, based on nutritional component and based on its functional use.

1) Classification based on consistency:

Culture media are liquid, semi-solid or solid and biphasic.

A) Liquid media: These are available for use in test-tubes, bottles or flasks. Liquid media are sometimes referred as “broths”(e.g nutrient broth). In liquid medium, bacteria grow uniformly producing general turbidity. Certain aerobic bacteria and those containing fimbriae (Vibrio & Bacillus) are known to grow as a thin film called

‘surface pellicle’ on the surface of undisturbed broth. Bacillus anthracis is known to produce stalactite growth on ghee containing broth. Sometimes the initial turbidity may be followed by clearing due to autolysis, which is seen in penumococci. Long chains of Streptococci when grown in liquid media tend to entangle andsettle to the bottom forming granular deposits. Liquid media tend to be used when a large number of bacteria have to be grown. These are suitable to grow bacteria when the numbers in the inoculum is suspected to be low. Inoculating in the liquid medium also helps to dilute any inhibitors of bacterial growth. This is the practical approach in blood cultures. Culturing in liquid medium can be used to obtain viable count (dilution methods). Properties of bacteria are not visible in liquid media and presence of more than one type of bacteria can not be detected.

B) Solid media: Any liquid medium can be rendered by the addition of certain solidify ing

agents. Agar agar (simply called agar) is the most commonly used solidifying ag ent. It is anunbranched polysaccharide obtained from the cell membranes of some species of red algae such as the genera Gelidium. Agar is composed of two long-chain polysaccharides (70% agarose and 30%agarapectin). It melts at 95oC (sol) and solidifies at 42oC (gel), doesn’t contribute any nutritive property, it is not hydrolyzed by most bacteria and is usually free from growth promoting or growth retarding substances.However, it may be a source of calcium & organic ions

Most commonly, it is used at concentration of 1-3% to make a solid agar medium. New Zealand agar has more gelling capacity than the Japanese agar. Agar is available as fibres (shreds) or as powders.

C) Semi-solid agar: Reducing the amount of agar to 0.2-0.5% renders a medium semi-solid. Such media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains (U-tube and Cragie’s tube). Certain transport media such as Stuart’s and Amies media are semi-solid in consistency. Hugh &Leifson’s oxidation fermentation test medium as well as mannitol motility medium are also semi-solid.

D) Biphasic media: Sometimes,a culture system comprises of both liquid and solid medium in the same bottle. This is known as biphasic medium (Castaneda system for blood culture). The inoculum is added to the liquid medium and when subcultures are to be made, the bottle is simply tilted to allow the liquid to flow over the solid medium. This obviates the need for frequent opening of the culture bottle to subculture. Besides agar, egg yolk and serum too can be used to solidify culture media. While serum and egg yolk are normally liquid, they can be rendered solid by coagulation using heat. Serum containing medium such as Loeffler's serum slope and egg containing media such as Lowenstein Jensen medium and Dorset egg medium are solidified as well as disinfected by a process of inspissation.

2) Classification based on nutritional component:

Media can be classified as simple, complex and synthetic (or defined). While most of the nutritional components are constant across various media, some bacteria need extra nutrients. Those bacteria that are able to grow with minimal requirements are said to non-fastidious and those that require extra nutrients are said to be fastidious. Simple media such as peptone water, nutrient agar can support most non-fastidious bacteria. Complex media such as blood agar have ingredients whose exact components are difficult to estimate. Synthetic or defined media such as composition of every component is well known.

3) Classification based on functional use or application:

These include basal media, enriched media, selective/enrichment media, indicator/diff erential media, transport media and holding media.

A) Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar considered basal medium.

B) Enriched media: Addition of extra nutrients in the form of blood, serum, egg yolk e tc,

to basal medium makes them enriched media. Enriched media are used to gro w nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media.

C) Selective and enrichment media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

D) Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water are used to recover pathogens from fecal specimens.

E) Differential media or indicator media: Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Exmples: MacConkey's agar, CLED agar, TCBS agar, XLD agar etc.

F) Transport media:Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi- solid in consistency. Addition of charcoal serves to neutralize inhibitory factors. Cary Blair medium and VenkatramanRamakrishnan medium are used to transport feces from suspected cholera patients. Sach's buffered glycerol saline is used to transport feces from patients suspected to be suffering from bacillary dysentery.

G) Anaerobic media: Anaerobic bacteria need special media for growth because they Need

low oxygen content, reduced oxidation – reduction potential and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced. Robertson cooked meat that is commonly used to grow Clostridium spps medium contain a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin. Methylene blue or resazurin is an oxidation- reduction potential indicator that is incorporated in the thioglycollate medium. Under reduced condition, methylene blue is colourless.

Preparation and preservation

Care must be taken to adjust the pH of the medium before autoclaving. Various pH indicators that are in use include phenol red, neutral red, bromothymol blue, bromocresol purple etc. Dehydrated media are commercially available and must be reconstituted as per manufacturers'recommendation. Most culture media are sterililized by autoclaving. Certain media that contain heat labile components like glucose, antibiotics, urea, serum, blood are not autoclaved. Thesecomponents are filtered and may be added separately after the medium is autoclaved.Certain highly selective media such as Wilson and Blair's medium and TCBS agar need not be sterilized. It is imperative that a representation from each lot be tested for performance and contamination before use. Once prepared, media may be held at 4-5oC in the refrigerator for 1-2 weeks. Certain liquid media in screw capped bottles or tubes or cotton plugged can be held at room temperature for weeks.

Preparation of McConkey Agar:

For 1000 ml preparation

- 20 gm. Peptone
- 2o gm. Agar

- 20 gm. Lactose
- 5 gm. Sodium Taurocholate

Add 1ml of Neutral Red as an indicator

Final pH should be 7.3.Autoclave at 121^{o}c for 15 minutes.

Lactose Fermenter: Pink e.g. E.coli.

Non Lactose Fermenter: Transparent e.g. Pseudomonas

Late Lactose Fermenter: Light Pink e.g. Acinetobacter

	Name	Age/Sex	MRD NO.	Requisition Type	Result
Case 1	Manzoor	38/M	130002245	IPD	Non Lactose Fermenter
Case 2	Lateef	36/M	130002776	OPD	Lactose Fermenter

Collection

Depending on the type of disease, various specimens are submitted to the laboratory. These include blood, wound, exudate, throat swabs, urine, spinal fluid, faecal material and others. However, the laboratory technician is still expected to be knowledgeable about which specimen are appropriate for a given disease, how those specimens should be collected and how they should be transported to the laboratory. One should always bear in mind that 'a bad specimen cannot yield good results.' If any inadequate or inappropriate specimen has been received in the laboratory, it is preferable to reject the specimen rather than to provide inaccurate results later. However, the technician must confer with the microbiologist before rejecting any valuable specimen.

Bacteriology

Gram Staining:

Principle:

The air dried fixed smear of bacteria, picks up stain and looks purple, when treated with crystal violet stain and iodine. Iodine enhances the staining reaction between the dye and the internal cellular constituents of bacteria. The alcohol decolourizes Gram negative bacteria while Gram positive bacteria retain the color. Counterstaining with safranin stains the Gram negative bacteria red. Under oil immersion microscopic examination, Gram negative organisms appear red e.g. E.coli, Salmonella etc. and Gram positive organism appear purple in color e.g. Staph. Aureus, Strep. Pyogenic etc.

Procedure:

- Prepare a smear using culture/sample
- Air dried the smear and heated it so that it is fixed
- Poured crystal violet solution for 1 minute and then rinsed in water
- Poured Gram's Iodine for 1 minute
- Wash with water
- Decolorize with Acetone for 30 seconds
- Wash with water and counterstain with Safranin for 30 seconds
- Wash in water and air dry
- Observe under microscope

Results:

	Name	Age/Sex	MRD NO.	Requisition Type	Result
Case 1	Manzoor	38/M	130002245	IPD	Gram Positive
Case 2	Lateef	36/M	130002776	OPD	Gram Negative

AFB Staining (ZN Staining)

Principle: A few species of bacteria in the genera Mycobacterium and Nocardia, and the parasite Cryptosporidium do not readily stain with simple stains. However, these microorganisms can be stained by heating them with carbol-fuchsin. The heat drives the stain into the cells. Once the microorganisms have taken up the carbolfuchsin, they are not easily decolorized by acid-alcohol, and hence are termed acid-fast. This acid-fastness is due to the high lipid content (mycolic acid) in the cell wall of these microorganisms. The Ziehl-Neelsen acid-fast staining procedure (developed by Franz Ziehl, a German bacteriologist, and Friedrich Neelsen, a German pathologist, in the late 1800s) is a very useful differential staining technique that makes use of this difference in retention of carbolfuchsin. Acid-fast microorganisms will retain this dye

and appear red. Microorganisms that are not acid-fast, termed non-acid-fast, will appear blue or brown due to the counterstaining with methylene blue after they have been decolorized by the acid-alcohol. A modification of this procedure that employs a wet-ting agent rather than heat to ensure stain penetration is known as the Kinyoun staining procedure (developed by Joseph Kinyoun, German bacteriologist, in the early 1900s).

Procedure:

- Prepare smear on the slide and heat fix.
- Add boiling CorbolFuchsin on slide.
- Wait for 5-10 minutes and then wash.
- Add 3% Acid Alcohol on slide for 5 minutes.
- Add 20% Sulphuric acid for decolourization for 4 minutes.
- Wash with water and add Methyl blue for 30 seconds.
- Rinse with water.
- Air dry and observe under microscope.

Results:

	Name	Age/Sex	MRD NO.	Requisition Type	Result
Case 1	Manzoor	38/M	130002245	IPD	AFB Positive
Case 2	Lateef	36/M	130002776	OPD	Negative

Biological Culture Method:

Methods of culturing bacteria vary and depend on the types of media being inoculated. Whatever medium is used, an aseptic technique must be used for two reasons.

- To prevent contamination of cultures and specimens in order to

avoid interference with the interpretation of culture results.

- To prevent contamination of the work area.

In order to achieve these two objectives, the lab worker should;

- Flame wire loops, straight wires and forceps before and after use.
- Flame the necks of the specimen bottles, culture tubes, and test tube after removing and replacing caps or plugs.
- Not let bottle tops or caps touch an unsterile surface.
- Always inoculate culture media before making smears
- Use the safety cabinet when treating hazardous pathogens.
- When sterilizing a wire loop, hold it in the blue part of flame with the loop part facing down. This is to ensure quick and proper sterilization of the wire loop and prevent sputtering material from falling on hand.

Different methods

1. Plate culture
2. Tube culture
3. Slope culture
4. Deep culture
5. Stab culture

PROCEDURE(Plate culture):

1. Collect the equipment's required for processing the urine specimen for culture.

2. After receiving the urine sample in the laboratory. We check the label on the urine container and make sure that it is similar with the requisite form. If there is any discrepancy we reject the sample.

3. Mix the urine specimen by inverting, Sterile the loop, and allow it to cool.

4. Open the urine container in front of flame and pick up loop full of inoculum on to

the Blood agar plate and then on McConkey agar plate and make primary inoculum and go for streaking i.e. primary streaking , secondary and finally tertiary streaking.

5. Incubate the inoculated plate in an incubator at 370c for overnight (optimum temperature for bacterial growth).

6. Next morning see the plate for bacterial growth, if no growth is present culture is sterile; if present go for identification and colony count.

7. For identification we make smear, and perform Gram stain.

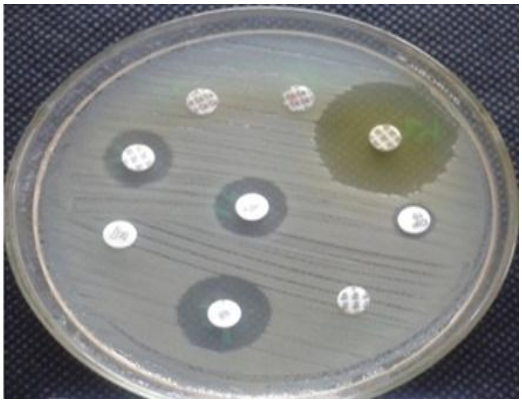
8. Perform Biochemical tests and simultaneously perform antimicrobial sensitivity testing using Kirby Bauer's disk diffusion method on MullerHinton agar plate.

9. Incubate plate at 370c in an incubator for overnight and next day see the sensitivity pattern of the organism against their respective antibiotic panel.

Result:

	Name	Age/Sex	MRD NO.	Requisition Type	Microscopy/Result
Case 1	Manzoor	38/M	130002245	IPD	Pus 15-20,Bacteria ++/Sterile
Case 2	Lateef	36/M	130002776	OPD	Pus 20-25,Bacteria ++/Growth of E.Coli>100000 cfu/ml

Antibiotic Sensitivity Pattern



SUMMARY ON MY PROJECT REPORT

This project Report is based on mostly positive patient only which has performed by me and I have mention short description laboratory work which is done by me in laboratory during training session in Medinova Diagonostic & Clinical Laboratory J&K. There are following department in the hospital in which I have worked like Pathology it has two sections (Histopathology & Hematology), Biochemistry, &Microbiology. The training period was of 4 month approximately, in this period of 4 months I worked in all the three departments (Pathology, Biochemistry,& Microbiology) on rotation basis, my training session was starting from Ist jan 2015 in histopathology of the pathology department it was having two sections(Histopathology & Hematology) for 1st fifteen days in this session of training I worked in different sections of histopathology and cytology starting from reception, tissue processing, block preparation and also perform staining like (H &E,PAP,MGG).

In the histopathology department these work is done by me Block preparation, HematoxylinStaining, PAPStaining. From 21stJan I was shifted to the hematology section, in this session I worked in the different sections of hematology lab like sample receiving and entering in the log book ,processing and examine of the urine specimen, coagulationprofile, blood grouping and CBC and reporting.

Then from 11th feb-13 to 17th march-15, I was send to the Biochemistry department in this session of training I worked in different sections of the biochemistry lab like sample receiving and entering in the log book and then in the serum separation and then processing through different analyzers and reporting.

And from 18th march to 20th april-15, I was send in the microbiology department in this session of training I worked in the different sections of the microbiology lab the sections which include in microbiology department are media room, in this section all the media which are used in the microbiology department is prepared in this room. Thencomes the collection room in this room all the samples were collected and received in this room. Then there is

bacteriology section in these different types of staining like ZN staining& Gram staining, urine culture and antibiotic sensitivity test was performed. The next section was of serology in this section different investigation was performed like anti-HIV trilling cassette test for AIDS, stained salmonella antigens for WIDAL(slide test),RF latex agglutination slide test, one step rapid test for Hepatitis B surface antigen. Then was the immunology section in this section HCV was performed on ELISA technique.

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