

A
On Job Training Report
On
PATHOLOGY LABORATORY
Completed at
Nishkarsh Pathology laboratory, Pandharpur

By
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Part I Semester II
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2024-25





Shri Swami Vivekanand Shikshan Sanstha's
VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)
PG Department of Microbiology



**CERTIFICATE
OF
"ON JOB TRAINING"**

This is to certify that Miss. Bhinge Revati Gajanan (Exam seat no. 1119130) has satisfactorily carried out the required practical work prescribed by the BoS Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) for M.Sc. - Part- I Semester II course in On Job Training (Sub code – OJT20MIC21) and this report represents her Bonafide work in the year 2024 - 2025

Place: Kolhapur

Date: 15-04-25

Karande
25/04/25
Banode
25/04/25
Examiner

Smali
OJT In charge

Ganpals
Head
VC HEAD
DEPARTMENT OF MICROBIOLOGY
VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)

DECLARATION

I hereby declare that I have successfully completed the On Job Training program at Nishkarsh Pathology Laboratory Pandharpur. I acknowledge that skills acquired during this training program are valuable to me and will contribute to my professional development.

I express my gratitude to Mrs. Prajakta Prasad Khadilkar of Industry, Designation, Name of Industry and the whole training team for their support and guidance throughout the training.

Date: 15-04-25

Place: Kolhapur

Name of student

Miss. Bhinge Revati Gajanan



ACKNOWLEDGEMENT

At this juncture where the herculean task is nearing its pinnacle, science deems it a pleasure to look back and acknowledge efforts and support of all kith and kin that helped with zeal to turn a distant dream of an industrial training into reality.

We are extremely thankful to Dr. S. D. Mali/ Dr. K. K. Bhise, Assistant Professor, PG Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute), project guide for her valuable guidance and mentorship throughout this project work given to us during the study.

We are indeed grateful to Head Dr. T. C. Gaupale, Coordinator Ms. V. V. Misal, PG Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) for their kind co-operation and valuable support and we are also thankful to all the staff members of our department for their direct and indirect support.

We are thankful to Principal Dr. R. R. Kumbhar, for his kind co-operation and valuable support.

Also, we sincerely thank our parents for helping us in all aspects to complete the project work. Finally, we would like to appreciate our friends, colleagues for their direct and indirect contribution.

Date: 15-04-25

Place: Kolhapur

Name of student

Miss. Bhinge Revati Gajanan

INTERNSHIP UNDERTAKING

1. Student Name	Bhinge Revati Gajanan
2. Current Address	Akshay park, backside of collector office, kolhapur
3. Residence Address	Govindpura , Pandharpur , Dist – Solapur
4. Email id	revatjbhinge@gmail.com
5. Mobile Nos.	7498173009
9. Internship /Area (Company/Institute)	Nishkarsh Pathology laboratory, Shivaji Chawk , Pandharpur

I confirm that I agree with the terms, conditions, and requirements of the Internship Policy

Student Signature: *RG Bhinge*

Date: 14-12-2024

I confirm that the student has attended the internship orientation and has met all paperwork and process requirements to participate in the internship program, and has received approval from his/her mentor.

Gajanan
Sign of Head of the Department: **VC HEAD
DEPARTMENT OF MICROBIOLOGY
VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)**

Date: 14-12-2024

ATTENDANCE SHEET

Name and Address of the Company/ Institute/Organization: Nishkarsh Pathology laboratory,
Shivaji Chawk, Pandharpur

Email Id: prajakta1398@gmail.com

Name of Supervisor: Mrs. Prajakta Prasad Khadilkar

Name of the Student	Bhinge Revati Gajanan
Roll Number	5403
Name of Course	MSC Microbiology
Date of Commencement of Training	16 Dec 2024
Date of Completion of Training	5 Jan 2025

Month and Year: Dec 2024 – Jan 2025

Sr. No	Date	Day	Time	Work done	Sign
1.	16-12-2024	First	9 AM TO 3 PM	Introduction about instruments and laboratory	RGBhinge
2.	17-12-2024	Second	9 AM TO 3 PM	Precautions in laboratory	RGBhinge
3.	18-12-2024	Third	9 AM TO 3 PM	Blood sample collection from patient	RGBhinge
4.	19-12-2024	Fourth	9 AM TO 3 PM	Blood sample collection from patient	RGBhinge
5.	20-12-2024	Fifth	9 AM TO 3 PM	Work in department of Clinical Pathology	RGBhinge
6.	21-12-2024	Sixth	9 AM TO 3 PM	Work in department of Clinical Pathology	RGBhinge
7.	22-12-2024	Seventh	9 AM TO 3 PM	Work in department of Clinical Pathology	RGBhinge
8.	23-12-2024	Eighth	9 AM TO 3 PM	Work in department of Biochemistry	RGBhinge
9.	24-12-2024	Nineth	9 AM TO 3 PM	Work in department of Biochemistry	RGBhinge
10.	25-12-2024	Tenth	9 AM TO 3 PM	Work in department of Biochemistry	RGBhinge
11.	26-12-2024	Eleventh	9 AM TO 3 PM	Work in department of Hematology	RGBhinge
12.	27-12-2024	Twelfth	9 AM TO 3 PM	Work in department of Hematology	RGBhinge
13.	28-12-2024	Thirteenth	9 AM TO 3 PM	Work in department of Hematology	RGBhinge
14.	29-12-2024	Fourteenth	9 AM TO 3 PM	Work in department of Serology	RGBhinge
15.	30-12-2024	Fifteenth	9 AM TO 3 PM	Work in department of Serology	RGBhinge

16.	31-12-2024	Sixteenth	9 AM TO 3 PM	Work in department of Serology	P. G. Phinse
17.	1-1-2025	Seventeenth	9 AM TO 3 PM	Work in department of Microbiology	P. G. Phinse
18.	2-1-2025	Eighteenth	9 AM TO 3 PM	Work in department of Microbiology	P. G. Phinse
19.	3-1-2025	Nineteenth	9 AM TO 3 PM	Work in department of Microbiology	P. G. Phinse
20.	4-1-2025	Twentieth	9 AM TO 3 PM	How to make report	P. G. Phinse
21.	5-1-2025	Twenty-first	9 AM TO 3 PM	Report analysis	P. G. Phinse



Certificate

This is to certify that Ms Bhinge Revatl Gajanan has worked as a

Trainee Laboratory Technician from 16/12/2024 to 05/01/2025.

Her work has been satisfactory.


Dr Anil Joshi

Pandharpur

05/01/2025

प्राथमिक पथक, लॅबोरेटरी
शिवाजी चौक, पंढरपूर.
☎ २२२९९४ / २२२९९२

Nishkarsh
PATHOLOGY
LABORATORY

'NACO/SACS' designated & MSACS Approved HIV Counselling & Testing Center

Jhamata Vyapar Sankul First Floor, Purv Bhag, Opp. Shivaji Statue, Pandharpur - 413304. Ph.: 02186-222992, 222994

SUMMARY OF TRAINING REPORT

This report describes a brief description of the work that has been carried out by me in the laboratory during training at Super Religare Laboratory (SRL). I have been working in laboratory during my training period from 16th December 2024 to 05th of January 2025. I have worked in five department and this department is clinical pathology, biochemistry, hematology, serology, microbiology.

In Clinical Pathology I have learn how to operate (Clinitek 500) a semi-automated urine chemistry analyzer instrument which gives numbers of the tests Glucose, Bilirubin Ketone, Protein, Urobilinogen, Nitrite, Leukocytes, colure of the urine and pH etc. I have also examined stool slides and semen where I observed some human parasites and abnormal immotile sperms.

In Biochemistry in section was too fully automated machine (DADE DIMENSION) which give the result Glucose, Uric acid, Cholesterol and Triglyceride.

In Hematology I have learned how to operate these machines (CBC LH750, ESR analyzer, Coagulation profile test). The test which conduct in this machines these are RBC, WBC, Platelets and clotting factors,

In Serology there was most of work done by manually but most of I have used readymade kit which provided by Manufacturer Company. These test were WIDAL, ASO, VDRL and free testosterone.

In Microbiology I have learned about staining, culture of blood, body fluids and there was (Vitek) which was automated and give the sensitivity of antibiotics and presence of different bacteria. I have mentioned in this report.

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CLINICAL PATHOLOGY

Introduction:

It is a medical specialty that is concerned with the diagnosis of disease based on the laboratory analysis of bodily fluids, such as urine, semen, and stool

Types of sample

1. Urine
2. Stool
3. Semen



Clinitek 500 Urine analyzer

Principles:

The reaction of Siemens Multistix 10 SG test strips depends on color development as an indicator of the concentration of the following test reactions.

Procedure urine examination

Routine (complete) Examination of Urine is divided in three parts:-

- A. Physical/Gross Examination.
- B. Chemical Examination.
- C. Microscopic Examination.

A. PHYSICAL EXAMINATION OF URINE DETERMINATION

Determination	Normal Finding	Abnormal	Pathologic
1. Volume of Urine	50 to 200 ml	>500 ml	Diabetes us, Polyuria
		<20 ml	Oliguria, Anuria
2. Color of Urine	Pale Yellow	Dark Yellow	Hepatic and post hepatic condition
	White	Redish	Chyluria, Hematuria
		Black Urine	Alkaptonuria
		Dark yellow	Biliverdin present
3. Appearance of Urine	Usually clear	Turbid	Presence abnormal Leukocytes,
		Milky	Chyle
4. Reaction	Usually acidic PH 4.88 to 7.5	PH less than 4.8 More, acidic Urine	Fever, Ketosis
	PH more than 7.5 Alkaline Urine		Sever Vomiting,
5. Odor of Urine	Aromatic	Fruity	Acidosis, Ketosis
		Ammonical	Cystitis
		Foul smelling	infection Urinary
6 Specific gravity of Urine	Varies from 1.003 to 1.060	Low Sp. Gravity	Chronic nephritis & diabetes insipid us
		High Sp. Gravity	Diabetes insipidus fever, Acute nephritis

Normal ranges of physical examination

TEST	ABBREVIATI ON	UNITS	NORMAL RANGES
Glucose	GLU	mg/dL	NEGATIVE
Bilirubin	BIL		NEGATIVE
Ketone	KET	mg/dL	NEGATIVE
Specific Gravity	SG		1.016-1.022
PH	PH		5.0-8.0
Protein	PRO	mg/dL	NEGATIVE
Urobilinogen	URO	E.U./dL	0.2-1.0
Nitrite	NIT		NEGATIVE
Blood	BLO		NEGATIVE
Leukocytes	LEU		NEGATIVE

B. CHEMICAL EXAMINATION:

1. Glucose.
2. Proteins

1. SUGAR (GLUCOSE) TEST ("BENEDICT'S QUALITATIVE TEST")

Principle:

Urine glucose reduces cupric ions present in the reagent to cuprous ion, Alkaline medium is provided to the reaction by sodium carbonate present in the reagent the original color change blue to green, yellow, orange and red A/C to concentration glucose.

Procedure of glucose

- 1) Take 5ml of Benedict's reagent in the test tube.
- 2) Add 8 drops of urine.
- 3) Boil for 2 minute and allow cooling under tap water.

Observation & Result:

Blue clear	- NEGATIVE
Green, no ppt	- Trace
Green with ppt	- +
Brown with cloudy	- ++
Orange with cloudy	- +++
Red with cloudy	- ++++

Disease - Hyperglycemia, Renal glycosuria,

2. Albumin Protein

Principle:

Sulphosalicylic acid solution (3%) precipitates any protein in the urine specimen irrespective of the type albumin or Bence jones. It is an anion precipitant that works by the neutralization of the protein cation.

Pathogenic: Nephritic syndrome.

3. Microscopic examination of urine

In microscopic, I examined the various cells likes Pus cells, RBCs, Epithelial cells, Triple phosphate Calcium oxalate. Cholesterol and Uric acid.

BIOCHEMISTRY

INTRODUCTION:

Clinical Biochemistry deals with the biochemistry laboratory applications. To find out Cause of disease The chemical constituent of various body fluid such as Blood, Urine, CSF and other body fluid like are analyzed in clinical biochemistry laboratory. The Biochemistry test are very useful to determine the severity of disease of many organ. The Clinical biochemistry tests in relation to the various clinical conditions.

1. The cause of disease
2. Screen assay diagnosis.
3. Suggested effective treatment.
4. Monitoring process of a pathological condition
5. Help in assessing response to therapy

PRINCIPLE:

The Principle of this instrument is based on Lamberts and Beers law. The optical density (O.D) is directly proportional to the concentration of solution and the thickness of the Cuvette.



Biochemistry Analyzer

NAME OF THE TEST:

1. Glucose

- . Fasting blood sugar
- . Random blood sugar
- . Postprandial blood sugar

2. Renal function test (RFT)

3. Liver Function Test (LFT)

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

4. Lipid Profile.

- . Cholesterol
- . Triglyceride

GLUCOSE

Principle

UV test enzymatic reference method with hexokinase. Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP. Glucose + ATP Hexokinase → G-6-P + ADP. Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

Procedure

Separate the serum or plasma sample from the test tube with the help of micro pipette. Take the sample in a cuvette. Give the command to the analyzer and select the tests. Press ok. Then place the cuvette in the analyzer. Analyzer gives result automatically.

Normal range of Glucose

Fasting:	Postprandial	Random
70-110 mg/dl	70-150 mg/dl	100-150 mg/dl 50

Case study 1

Name - VINEET KUMAR

Age/sex -41/male

Sample: - Fasting plasma

Result obtained -140 mg/dl

Interpretation: The blood glucose level in the patient is high which indicates hyperglycemia.

Case study 2

Name -Rahul

Age/sex -32/male

Sample -Random plasma

Result obtained -68 mg/dl

Interpretation: The blood glucose level in the patient is low which indicates hypoglycemia

Clinical significance

Hyperglycemia	Hypoglycemia
Diabetes mellitus	Overdose of insulin
Hyperactivity of thyroid, adrenal, pituitary gland	Hypo activity of thyroid, adrenal, or pituitary gland
	Glycogen storage disease in which there is deficiency of G-6-phosphat

RFT (RENAL FUNCTION TEST)

Blood urea nitrogen (BUN)

Principle:

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



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.



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

Normal range : -7-10 mg/dl

Case Study: 1

Name: - Shanoo Jah
Age/sex: - 21/female
Sample: - Serum
Result obtained: - 6 mg/dl

Interpretation: The blood urea nitrogen level in the patient is low.

Case Study: 2

Name: - Manshi Kumari
Age/sex: - 34/female
Sample: - Serum
Result obtained: - 26 mg/dl

Interpretation: the blood urea nitrogen level in patient is high.

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 perianal azotemia (e.g. shock), post renal azotemia, GI bleeding, and a high protein diet. Some causes of decreased values for urea nitrogen include pregnancy, severe liver insufficiency, over hydration and malnutrition.

LIVER FUNCTION TEST (LFT)

Introduction

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time. Aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), measure the concentration of intracellular hepatic enzymes that leaked into the circulation and serve as a marker of hepatocyte injury.

TOTAL BILIRUBIN

Principle:

Diazotized Sulfanilic acid is formed by combining sodium nitrite and sulfanilic acid at low pH. The sample is diluted in 0.05M Hydrochloric acid. A blank reading is taken to eliminate interference from non-bilirubin pigments. Upon addition of the diazotized sulfanilic acid, the conjugate bilirubin is converted to diazo-bilirubin, a red chromosphere which absorbs at 540nm.

Normal range: 0.20-1.00mg/dl

Case study-1

Name	-Pankaj kale
Age/sex	-38/male
Sample	-serum

Result obtained: - 1.53 mg/dl

Interpretation: Total Bilirubin level in patient's serum is high.

Clinical Significance

High levels of bilirubin in the blood may be caused by:

Some infections, such as an infected gallbladder.

Some inherited diseases, such as Gilbert's syndrome, a condition that affects how the liver processes bilirubin. Although jaundice may occur in some people with Gilbert's syndrome, the condition is not harmful.

Diseases that cause liver damage, such as hepatitis, cirrhosis, or mononucleosis.

Diseases that cause blockage of the bile ducts, such as gallstones or cancer of the pancreas.

SGPT (Serum glutamate Pyruvate Transaminase)

(Also called ALT (Alanine Transaminase))

Principle:

Alanine aminotransferase catalyzes the transamination of L-alanine to α -ketoglutarate, forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced nicotinamide-adenine dinucleotide (NADH). The change in absorbance is directly proportional to the ALT activity and is measured using a dichromatic (340, 700 nm) rate technique

L-Alanine + α -ketoglutarate ALT pyruvate + L-glutamate

Pyruvate + NADH + H⁺ LDH L-lactate + NAD⁺

Normal range: 30-65 U/L

Case study:

Name: - Neha mane

Age/sex -21/female

Sample -serum

Result obtained: -116 U/L

Interpretation: The ALT level in patient's serum is high

Clinical Significance

High levels of ALT may be caused by:

- Liver damage from conditions such as hepatitis or cirrhosis.
- Lead poisoning.
- Exposure to carbon tetrachloride.
- Decay of a large tumor (necrosis).

SGOT (Serum Glutamate Oxaloacetate Transaminase)

Also called AST (Aspartate Transaminase)

Principle:

Aspartate aminotransferase catalyzes the transamination of L-aspartate to α -Ketoglutarate, forming L-glutamate and oxaloacetate. The oxaloacetate formed is reduced to malate by malate dehydrogenase (MDH) with simultaneous oxidation of reduced nicotinamide-adenine dinucleotide (NADH). The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to the AST activity and is measured using a dichromatic (340, 700 nm) rate technique.

L-Aspartate + α -ketoglutarate $\xrightarrow{\text{AST}}$ oxaloacetate + L-glutamate

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

Normal range: 15-37 U/L

Case study-1

Name -Neha mane

Age/sex -21/female

Sample -serum

Result obtained: - 52 U/L

Interpretation: The AST level in patient's serum is high.

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

LIPID PROFILE

CHOLESTEROL

Principle

Cholesterol esters are hydrolyzed by cholesterol ester hydrolase to produce free cholesterol and fatty acids. The free cholesterol produced and pre-existing one is oxidized by cholesterol oxidase to cholestenone-4-en-3-one and hydrogen peroxide. Hydrogen peroxide thus formed is used to oxidize N, N diethylaniline- 4-aminoantipyrine to produce a chromosphere that absorbs at 540 nm. The absorbance due to oxidized N, N diethyl aniline- 4-aminoantipyrine is directly proportional to the total cholesterol concentration and is measured using a polychromatic (452, 540,700 nm) end point technique.

Normal range: 0-200mg/dl

Case study

Name: -Naina Rnjane

Age/sex: -21/female

Sample: - serum

Result obtained: -236 mg/dl

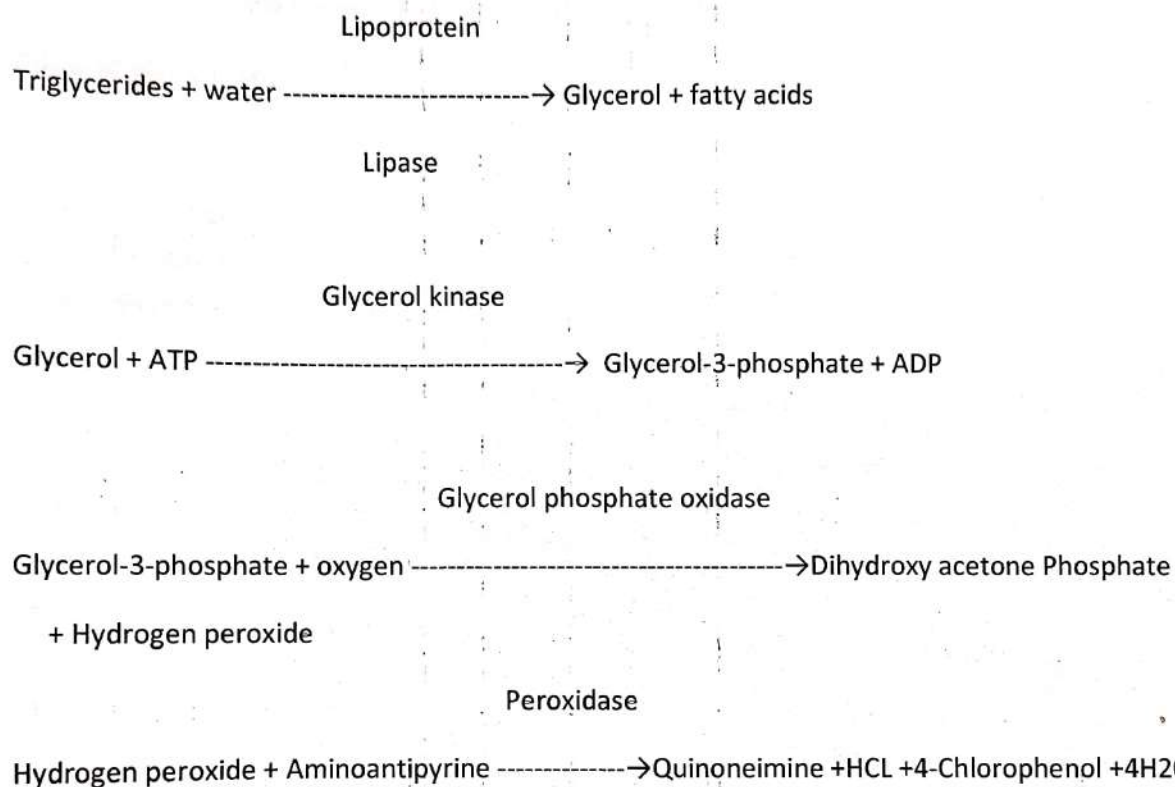
Interpretation: The cholesterol level in patient's serum is high.

Clinical Significance

Elevated levels of serum cholesterol are associated with atherosclerosis, nephritis, diabetes mellitus, Obstructive jaundice, Biliary cirrhosis, lipoproteinemias, and myxedema. Decreased level in cholesterol is associated with severe infection, severe anemia, and malnutrition.

TRIGLYCERIDES

Principle



The change in absorbance due to the formation of Quinonimine is directly proportional to the total amount of glycerol and its precursors in the sample and is measured using a dichromatic (510, 700 nm) endpoint technique.

Normal range: 15-1000mg/dl.

Case study

Name: - Mohan shinde

Age/sex: - 34/male

Sample -Serum

Result obtained: 156 mg/dl

Interpretation: the triglycerides level in patient is high.

Clinical significance:

TG level decreases in:

- Liver disease
- Cerebral infarction,
- Hyper parathyroidism

HEAMATOLOGY

INTRODUCTION

Hematology is the study of blood, blood components, and blood disorders it involves Studying the anatomy and physiology of blood cells and other cells that compressed Blood like Red blood cells White blood cells Platelets and hemoglobin.

1. Analysis of blood concentration, structure and function of the cells and their precursors In the bone marrow.
2. Analysis of chemical constituents of plasma or serum intimately, linked blood cells Structure and function. Study of function of the platelets and proteins involved in blood coagulation.

NAME OF THE INSTRUMENT:

- LH 750 (For detection of Hb, Platelets and CBC
- Centrifuge
- Wintrobe tube

NAME OF THE TEST:

1. Complete blood Count (CBC)
2. Erythrocyte sedimentation rate (ESR)
3. Blood grouping
4. Differential leukocyte count (DLC)
5. G-6-PD-1 6. Coagulation profile

Complete blood Count (CBC)

PRINCIPLE OF CBC ANALYSIS:

The Coulter method accurately counts and sizes cells by detecting and measuring changes in electrical resistance. When a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an Insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This cause is measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume.



CBC analyzer machine

PARTS OF THE INSTRUMENTS:

- Aperture Current.
- External electrode.
- Sample beaker.
- Aperture.
- Aperture tube.
- Blood cell suspension.

RESULTS

Aditya jain	Shreya navale	Normal range
WBC=3.8(L	WBC=15.2	4-11 cumm
NE=64.4%	NE=56.1	40-75%
LY=14.9%(L)	LY=42.6	20-45%
MO=17.2%(H)	MO=0.2	2-8%
EO=2.4%	EO=1.1	1-4%
BA=1.1%	BA=0.0	0-1%
RBC=4.07(L)	RBC=1.51	3-5 Lakh
HGB=12.2(L)	HGB=3.2	13-17 g/dl
HCT=37.1(L)	HCT=10.0	42-52%

MCV=91.1	MCV=66.2	80-100 f
MCH=30.0	MCH=20.8	27-32 Pg.
MCHC=32.9	MCH=31.5	32-36%
RDW=15.7%(H)	RDW=38.1	11-14%

1. Erythrocyte sedimentation rate

PRINCIPLE:

The red cells form Rouleaux, The settling/sedimentation of RBC's occur at a constant rate.

The individual cells also aggregates due to overcrowding, and get packed down on

The bottom of the tube.



ESR Analyzer

Reagents and Equipment:

1. Automated Analyzer
2. Westergren tube rack

3. Timer

4. 3.8% tri-sodium citrate

5. Test tubes

PROCEDURE:

- Take a clean dry centrifuge tube.
- Add 0.5ml of 3.8% sodium citrate.
- Add 2 ml blood sample into the tube and mix it.
- Fill the Westergren tube up to '0' mark.
- Pull the tube in vertical position on the stand.

Clinical Significance

ESR increased in:	ESR decreased in:
Chronic inflammations & infections Eg. TB	Polycythemia
Acute inflammations & infections	Sickle cell disease
Normal Pregnancy (Physiological)	Cryoglobinaemia

ABO BLOOD GROUPING BY

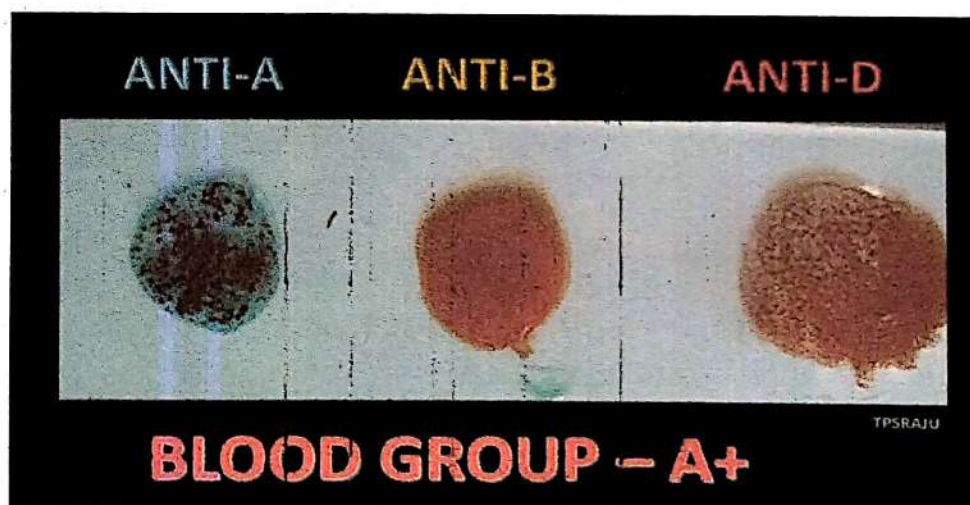
(Slide Method)

PRINCIPLE:

Serum of the specimen submitted is reacted with known A cells and B cells. Agglutination indicates presence of corresponding antisera in serum.

PROCEDURE:

1. Place 1 drop of anti-A and 1 drop of anti-B reagent separately on a labeled slide or tile.
2. Add 1 drop of 20% test red cell suspension to each drop of the typing antiserum (the suspension may be prepared by adding 20 parts of red cells to 80 part of normal saline).
3. Mix the cells and reagent using a clean stick. Spread each mixture evenly on the slide over an area of 10-15 mm diameter.
4. Tilt the slide and leave the test for 2 minutes at room temperature. Then rock again and look for agglutination.
5. Record the results.



Observation:

Reaction	Monoclonal Antibodies A	Monoclonal Antibodies B	Monoclonal Antibodies D	Result Blood Group
Agglutination	+	-	+	A Positive
Agglutination	+	-	-	A Negative
Agglutination	-	+	+	B positive
Agglutination	-	+	-	B negative
Agglutination	+	+	+	AB Positive
Agglutination	+	+	-	AB- Negative
Agglutination	-	-	+	O positive
Agglutination	-	-	-	O negative

blood grouping reaction

SEROLOGY

Introduction:

Serology is the study of immune bodies in human blood. These immune bodies are the product of the defense mechanisms against disease-causing organisms in the body. The principle involved with serology is the antibody-antigen response. The antigen actually comes first, in that the antigen is the substance which "provokes" the body to produce antibodies

The tests performed in serology lab:

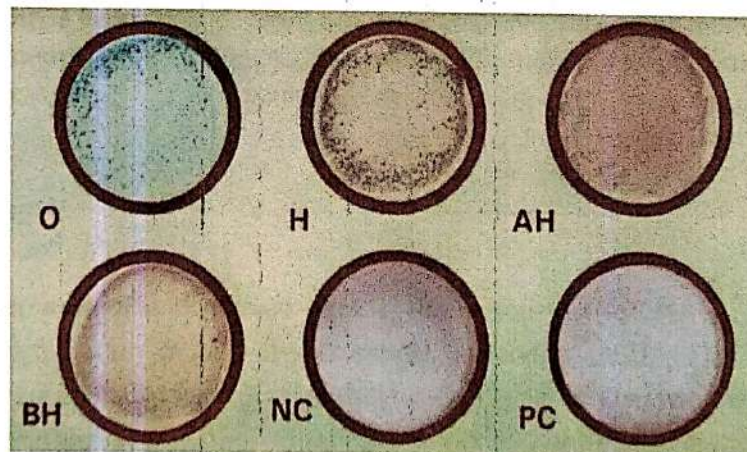
- WIDAL test
- ASO (Anti Streptolysin O)
- VDRL (Venereal disease research laboratory)

WIDAL TEST:-

WIDAL slide test provides a simple way of qualitatively and semi-quantitatively estimating the antibodies to *S.typhi* (O&H) and *S.paratyphi* (AH &BH). It is based on the principle of direct agglutination. When the patient's serum (containing antibodies to *S.typhi* & *S.paratyphi*)

Procedure of WIDAL test:

Take a clean glass slide add serum 1 drop in each four circle-add a drop of all 4 antigens in each circle. 1,2,3, and 4.



WIDAL reaction

RESULT:

If Agglutination titer if 1:80 or more is significant. An increase in titer, 4 to 5 days after the first test is suggestive of active Salmonella infection

CLINICAL SIGNIFICANCE:

S.typhi, S.paratyphi based on their antigenic structure are classified as 'O' (somatic) and 'H' (FLAGELLAR) Antigens. 'O' antigens of various species have common antigenic components. Hence only one antigen S- typhi O' is used in the routine test's antigen is species specific.

VDRL (Venereal disease research laboratory

PRINCIPLE:

Patients suffering from syphilis produce antibodies that react with Cardiolipin antigen in a slide flocculation test, which are read using a microscope.

Procedure:-

Add 50µl serum sample at RT → add 20 ul antigen and shake it mix with sticks rotate for 4min at 150rpm. And see the agglutination.

Results interpretation:

POSITIVE REACTION	POSITIVE REACTION	NEGATIVE REACTION
Marked and intense visible aggregates are seen. Serum sample is reactive.	Slight but definite small aggregates are seen. Serum sample weakly reactive.	The mixture remains in a smooth suspension with no visible aggregates. Serum is non-reactive.

ASO (Anti Streptolysin O) it is a rapid latex agglutination test for the qualitative and semi-quantitative determination of anti-Streptolysin-O antibodies (ASO) in serum. In infections caused by β -hemolytic streptococci, Streptolysin-O is one of the two hemolytic exotoxins liberated from the bacteria that stimulate production of ASO antibodies in the human serum.

PRINCIPLE:

The ASO is a rapid agglutination procedure for the direct detection and semi- quantitation (on slide) of anti-Streptolysin.

The antigen, a latex particles suspension coated with Streptolysin O agglutination in the presence of specific antibodies present in sera of patients with Streptococcal beta- hemolytic infection (Group A and C).

PROCEDURE

- Place 1 drop of serum sample on to the slide with the help of disposal serum dropper.
- Add 1 drop of ASO- Latex Antigen to the slide
- Mix properly with the applicator stick
- Rotate for 2min in a rotator
- Observe for agglutination

Positive Cases of patients

Name	Age/Sex	Result	ASO	VDRL	WIDAL
Shreya navale	23/F	Agglutination	+ve	-ve	-ve
Pragati waje	21/F	Agglutination	-ve	+ve	-ve
Disha walvekar	19/F	Agglutination	-ve	-ve	+ve

Patients positive cases

Principle

HIV antigens are immobilized on a porous immunofiltration membrane. Sample and the reagent pass through the membrane and are absorbed into the underlying absorbent. As the patients sample passes through the membrane, HIV antibodies, if present, bind to the Immobilized antigens. Conjugate binds to the Fc portion of the HIV antibodies to give distinct pinkish purple Dot (s) against a white background.

Procedure

- Add 3 drops of buffer solution to the center of the device.
- Hold the dropper vertically and add 1 drop of patient's sample (serum or plasma)
- Add 5 drops of buffer solution.
- Add 2 drops of liquid conjugate directly from the conjugate vial.
- Add 5 drops buffer solution and read the results

Result: - Report the result positive when both line is appeared control and HIV.

Microbiology

Introduction

Clinical microbiology is the branch of medical science that deals with the study of Microorganisms that infect humans, the disease they cause, their diagnosis prevention, and treatment.

Here microbiology department is divided into three sub departments.

1. Bacteriology department
2. Mycology department
3. Tuberculosis department

Types of sample received in Laboratory

- A. Urine (mostly received)
- B. Sputum sample
- C. Blood sample
- D. Stool sample
- E. Throat swab
- F. Water
- G. FNAC smear

Instruments:

1. Bactec system
2. Microscan
3. Microscope
4. Hot air oven
5. Incubator

Procedure for culture:

Urine, Stool, Body fluid, and CSF etc...

Take the sample

↓

And keep all equipment in the laminar air flow

↓

Inoculate the sample into the media and keep Inverted position



After cold keep the media into the incubator at RT for 24 hrs.

Result: - note down the result after 24 hrs. Or 48 hrs.

Microscopy:

BACTEC Wet mount is prepared. Single drop of urine is taken in a clean and dry slide, put on the coverslip and observe under the microscopy.

STAINING

Gram staining Principle	Acid staining Principle
<ul style="list-style-type: none">• Place the slide on the staining glass rods.• Cover the smear with crystal violet stain and leave for 1 minute.• Wash carefully under running tap water.• Flood the smear with the gram's solution and wait for one minute. Drain off the iodine.• Decolorize the smear with alcohol-acetone 7. (or rectified spirit) for 20-30 seconds (continue till purple stain just stops coming on the slide).	<ul style="list-style-type: none">• Prepare Smear from the sputum specimen on glass slide and fix it by heating.• Flood slide with Carbol-fuchsin stain, heat the slide gently with a flame for 5 minutes. Do not over heat the stain if necessary add carbol-fuchsin.• Rinse off the over stain under running tap water.• Decolorize with acid alcohol or 20% H₂SO₄ for about 1 minute or until no more color comes off.• Rinse again in running tap water.• Counter stain with methylene blue for 30 sec.• Examine under microscope with oil immersion objective.

Procedure

RESULTS

Gram stain	Acid fast AF
Yeast cells: Dark purple Epithelial cells: Pale red Nuclei of pus cell: Red Gram positive bacteria: Dark purple Gram negative bacteria: Pale to dark red.	Acid fast (AF) organisms – Bright red bacilli on blue background. Other organisms - Dark blue