A training Report on

Histopathological Tests

Submitted to

Atmiya University

In partial fulfillment of the requirements for the degree of

Bachelor of science in Biotechnology

By

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We express our deepest thanks to Dr. Pratyush Parmar for taking part in useful decision & giving necessary advices and guidance and arranged all facilities to make life easier. We choose this moment to acknowledge his contribution gratefully.

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PATHOLOGY LABORATORY
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EXPERIENCE CERTIFICATE

This is to certify that **Ms. Payal Sharma** studying in ATMIYA UNIVERSITY has taken training at Dr. Bhatt Pathology Laboratory, Delta Diagnostics Branch, Rajkot for the period of 125 hours. She has successfully completed training in Hematology, Biochemistry and Clinical Pathology. She is well versed with Phlebotomy process and Reception process.

Date: 20th JAN 2023

Place: Rajkot

Dr. Pratyush Parmar

DR. PRATYUSH PARMAR
M.B.B.S.; D.C.P.
Pathologist

DR. BHATT ★ PATHOLOGY LABORATORY ★

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Introduction

- With over 30 years of experience, Dr. Bhatt lab is the most trusted Lab of Saurashtra. Based in Rajkot, it is the first Lab to be NABL Certified in the region.
- Every Year, they perform more than 5,00,000 test on more than 2,50,000 Fully barcoded samples.
- They are trusted by More than 700 Doctors and more than 500 Labs in Saurashtra Kutch Region.
- Lab contains various departments like various departments like molecular pathology, histopathology, hematology, genetics, cytology, clinical pathology etc.
- They also gives facility of home collection of samples for patient's convinence.
- Lab contains team of various qualified doctors who makes procedures and results more reliable.
- Lab participates in extrernal quality control programmes with AIIMS and CMC Vellore.
- Dr. Bhatt lab works with automated procedures like sample barcoding, sample transport and modular testing samples to minimize human errors.
- Double checking of critical values with re-run is done before reporting.
- Lab works 24 x 7, 365 days.
- Lab follows "first in first out" and "stat" policy to ensure timely and unbiased reporting.
- Mission of Dr. Bhatt lab is to provide quality diagnostic services to patients, doctors and labs in Saurashtra-Kutch region by caring and empathizing with patients.
- Vission of the lab is to become leading diagnostic lab in Saurashtra-Kutch region, endorsed by doctors, preferred by labs and trusted by people.
- Dr. Bhatt lab works by 7 steps of sampling which are sample collection, sample transport,sample registration and labelling, sample processing and testing, sample rechecking, sample reporting and sample storage.

CBC Test

Introduction:

A CBC gives your provider a picture of your overall health. Using a small amount of blood, a CBC can help detect hundreds of conditions, disorders and infections. It allows your provider to monitor your health, screen for disease and plan and adjust treatment. The CBC machine is used to measure the number of RBCs, WBCs, and platelets in blood.

The amount of haemoglobin and hematocrit are also measured.

CBC machines are Classified based on number of types of WBC differentiated.

3-Part differential Cell Counter: reports only 3 types of WBCs (neutrophils, lymphocytes, and monocytes).

5-part Differential Cell Counter: can differentiate all WBC types (neutrophils, lymphocytes, basophils, eosinophils, and monocytes).

Principle :

The CBC generates accurate cell counts using the Coulter principle.

The Coulter principle states that particles passing through an orifice (along with an electrical current) will produce an increase in impedance, due to the displacement of electrolytes caused by the presence of the particle.

This change in impedance is proportional to the volume of the particle. The Coulter principle has been used for particle counting and sizing in a variety of fields, but one of the first and most impacted fields was hematology.

Requirements :

Blood sample, Antiseptic wipe, injection, Tourniquet, EDTA test tube, cell blood counter.

Procedure:

1.Clean the Skin with an antiseptic wipe and place an Tourniquet around patients upper arm to help the vein swell with blood.

2. Then a needle is inserted through the area of cleaned skin into the patients vein.

3.The blood is then pulled from the needle by a syringe and remove the Tourniquet.

4. The blood sample is taken into the EDTA tubes and label the sample.

5. Then the sample is taken for analysis in blood cell counter. The machine analysis the blood automatically and shows the Results.

Results:

In general, the reference ranges are:

White blood cells: 4,500 to 11,000 cells per microliter (cells/mcL)

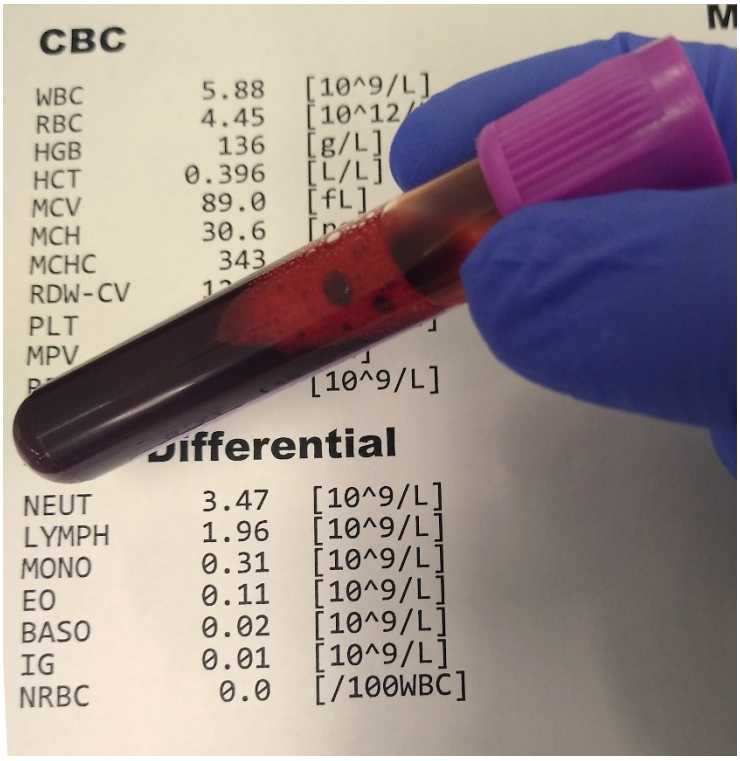
Red blood cells: 4.5 million to 5.9 million cells/mcL for men; 4.1 million to 5.1 million cells/mcL for women

Hemoglobin: 14 to 17.5 grams per deciliter (gm/dL) for men; 12.3 to 15.3 gm/dL for women

Hematocrit: 41.5% to 50.4% for men; 35.9% to 44.6% for women

Mean corpuscular volume: 80 to 96

Platelets: 150,000 to 450,000 platelets/mcL



Malaria Slide Test

Introduction:

Malaria is a life-threatening disease spread to humans by some types of mosquitoes. It is mostly found in tropical countries. It is preventable and curable.

Malaria is caused by the Plasmodium Parasite. The parasite can be spread to humans through the bites of infected mosquitoes (female Anopheles mosquito).

There are many different type of Plasmodium Parasite, but only 5 types cause Malaria in humans:

Plasmodium falciparum

Plasmodium vivax

Plasmodium ovale

Plasmodium malariae

Plasmodium knowlesi

Principle:

Malaria parasites can be identified by examining under the microscope a drop of the patient's blood, spread out as a "blood smear" on a microscope slide. Prior to examination, the specimen is stained (most often with the Giemsa stain) to give the parasites a distinctive appearance. This technique remains the gold standard for laboratory confirmation of malaria.

However, it depends on the quality of the reagents, of the microscope.

Requirements: blood sample, slides, microscope, needle, Giemsa stain

Procedure:

1.The second or third finger is usually selected or cleaned

2. punctured at the side of the ball of the finger

3. Gently squeeze toward the puncture site

4. Slide must always be grasped by its edges

5. Touch the drop of blood to the slide from below

6. Spread the first drop to make a 1 cm circle

7. Touch afresh drop of blood to the edge of another slide

8. wait for both to dry before fixing and staining

9.pull the drop of blood across the first slide in one motion

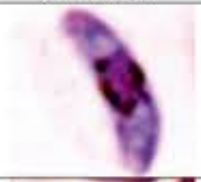
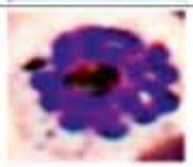
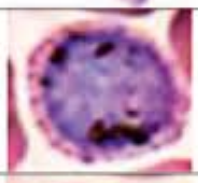
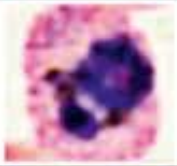
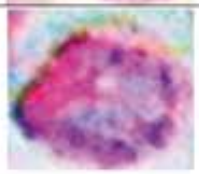
10. touch one drop of blood to clean slide

11. carry the drop of blood to the first slide and hold at 45 degree angle.

12. Prior to examination, the specimen is stained (most often with the Giemsa stain) to give the parasites a distinctive appearance.

13.malaria blood smear used to determine the species entire thin film should be examined about 20 to 40 minutes.

Result:

Species	STAGES			
	Ring	Trophozoite	Schizont	Gametocyte
P.falciparum				
P.vivax				
P.ovale				
P.malaria				

ESR Test

Introduction:

The erythrocyte sedimentation rate is a common hematology test that may indicate and monitor an increase in inflammatory activity within the body caused by one or more conditions such as autoimmune disease, infections or tumors. The ESR is not specific for any one disease but is used in combination with other tests to determine the presence of increased inflammatory activity. The ESR has long been used as a "sickness indicator" due to its reproducibility and low cost.

Principle:

When an anticoagulant is added to the blood and this well mixed venous blood is placed in a vertical tube, erythrocytes tend to settle towards bottom leaving clear plasma on top. This rate of sedimentation of red blood cells in a given interval of time is called erythrocyte sedimentation rate (ESR).

As the erythrocytes sediments, in a period of one hours, 3 stages can be observed.

Stage I: first 10 minutes:- It is initial period of aggregation during which rouleaux are formed and the sediment rate is low

Stage II: next 4o minutes:- It is a period of fast setting. Sedimentation occurs at a constant rate during this period

Stage III: next 10 minute or more:-The sedimentation again slows as it is the final period of packing of cells at the bottom of the tube.

RBCs typically fall at a faster rate in people with inflammatory conditions such as infections, cancer, or autoimmune conditions. These conditions lead to an increase in the number of proteins in the blood. This increase causes red blood cells to stick together (clump) and settle at a faster rate.

Requirements: Blood sample, wintrobe ESR tube, ESR stand, capillary pipette, centrifuge.

Procedure:

1.ESR should be done within 2 hours of the collection of the blood.

2.Mix the whole blood for at least 2 minutes on a rotator.

3.Take 0.2 mL of ESR solution + 1.8 mL of oxalate blood or blood in EDTA.

4.With a capillary pipette, Fill the Wintrobe ESR tube. (mix the blood thoroughly before filling the tube).

5.Mount in the ESR stand.

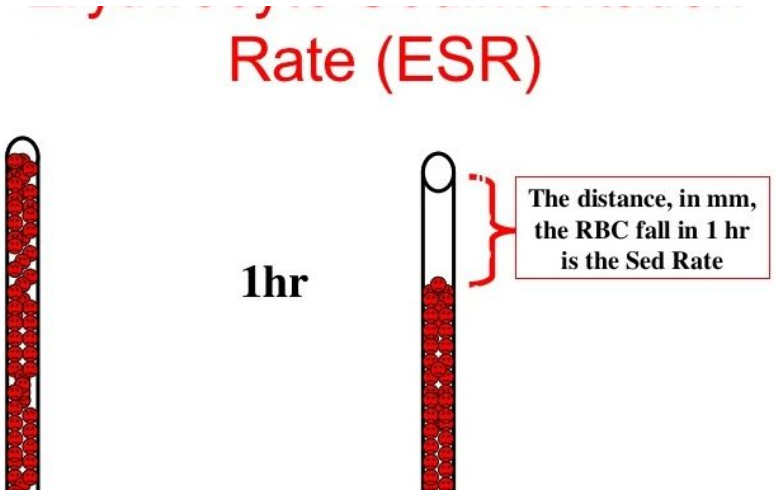
6.Start the clock for one hour.

7.Record the result after one hour.

8.That is the ratio of settled cells and above clear plasma.

9.The temperature should be kept between 20 to 25 °C.

Result:



A high ESR test result may be from a condition that causes inflammation, such as:

Arteritis, Arthritis, Systemic vasculitis,Polymyalgia rheumatica

A low ESR test result

Means your red blood cells sank more slowly than normal. This may be caused by conditions such as:

A blood disorder, such as:

Polycythemia, Sickle cell disease (SCD)

Leukocytosis, a very high white blood cell count (WBC)

If your ESR results are not normal, it doesn't always mean you have a medical condition that needs treatment. Pregnancy, a menstrual cycle, aging, obesity, drinking alcohol regularly, and exercise can affect ESR results.

HAEMOGLOBIN TEST:

Introduction:

A hemoglobin test measures the amount of hemoglobin in your blood. Hemoglobin is a protein in your red blood cells that carries oxygen to your body's organs and tissues and transports carbon dioxide from your organs and tissues back to your lungs.

If a hemoglobin test reveals that your hemoglobin level is lower than normal, it means you have a low red blood cell count (anemia). Anemia can have many different causes, including vitamin deficiencies, bleeding and chronic diseases.

If a hemoglobin test shows a higher than normal level, there are several potential causes — the blood disorder polycythemia vera, living at a high altitude, smoking and dehydration.

Principle:

The principle of this method lies in conversion of hemoglobin to cyanmethemoglobin by the addition of Potassium cyanide and ferricyanide whose absorbance is measured at 540 nm in a photoelectric calorimeter against a standard solution.

Requirements:

N/10 HCl-, Hemoglobin tube, Comparator

Stirrer**,** Blood sample

Needle for pricking fingertip,Glass slide

Spirit

Procedure:

- Add N/10 HCl to the hemoglobinometer tube up to its lowest mark i.e 2g% (If N/10 HCl is taken above the mark the color of undiluted solution is lighter than standard and if N/10 HCl is taken less then all the hemoglobin is not converted).
- Take blood up to 20 cu mm mark on the pipette and transfer it to the hemoglobinometer tube containing N/10 HCl.
- Leave the solution for 10 minutes (for the conversion of hemoglobin to acid hematin)
- After 10 minutes add distilled water drop by drop and mix it by stirrer until the color matches with the color of comparator. While matching the color glass rod should be removed from the solution.
- The lower meniscus of the solution should be taken as the result which expresses hemoglobin content as g%

If the hemoglobin is too low (less than 3g/dl) then 40µl blood is added to HCl upto 20 marks in the tube. Color is matched and result is halved

RESULTS:

The healthy range for hemoglobin is:

- **For men,** 13.2 to 16.6 grams per deciliter
- **For women,** 11.6 to 15 grams per deciliter

Healthy ranges for children vary with age and sex. The range for a healthy hemoglobin level may differ slightly from one medical practice to another.

URINE TEST

Introduction:

A urinalysis (also known as a urine test) is a test that examines the visual, chemical and microscopic aspects of your urine (pee). It can include a variety of tests that detect and measure various compounds that pass through your urine using a single sample of urine.

Healthcare providers often use urinalysis to screen for or monitor certain common health conditions, such as liver disease, kidney disease and diabetes, and to diagnose urinary tract infections (UTIs).

While several different aspects of your health can be tested with a urine sample, your healthcare provider will choose which tests to order under a urinalysis depending on your symptoms and situation. What tests are included in urinalysis?

- Color and appearance.(normal- shade of yellow)
- Chemical findings.(could be protein related)
- Microscopic findings.(Cells,Cell fragments,Urinary casts,Mucus.Bacteria or other germs,Crystals.)

Procedure: Cleanse the urinary opening. Women should spread the labia and clean from front to back. Men should wipe the tip of the penis.

- Begin to urinate into the toilet.
- Pass the collection container into your urine stream.
- Urinate at least 1 to 2 ounces (30 to 60 milliliters) into the collection container.
- Finish urinating into the toilet.
- Deliver the sample as directed by your health care provider.
- If you can't deliver the sample to the designated area within 60 minutes of collection, refrigerate the sample, unless your provider has told you otherwise.

Result:

Visual exam

A lab technician examines the urine's appearance. Urine is typically clear. Cloudiness or an unusual odor can indicate a problem, such as an infection. Protein in urine can make it appear foamy.

Blood in the urine can make it look red or brown. Urine color can be influenced by what you've just eaten or by certain drugs you're taking. For example, beets or rhubarb might tint your urine red.

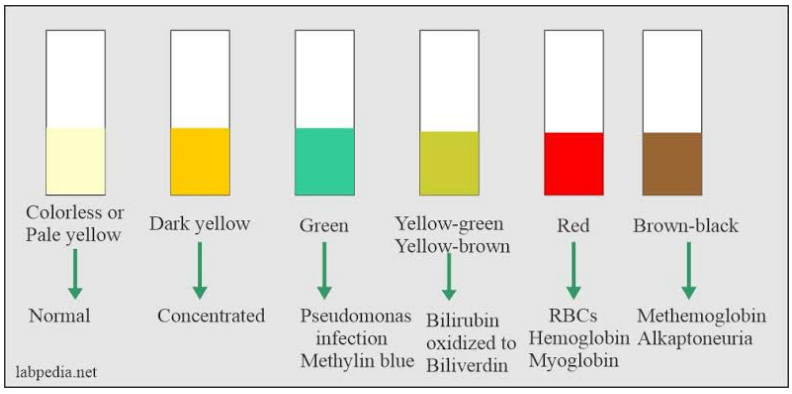
Dipstick test : A dipstick — a thin, plastic stick with strips of chemicals on it — is placed in the urine. The chemical strips change color if certain substances are present or if their levels are above typical levels. A dipstick test checks for:

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

bladder stones, kidney or bladder cancer, or blood disorders.

Microscopic exam: Sometimes performed as part of a urinalysis, this test involves viewing drops of concentrated urine — urine that's been spun in a machine — under a microscope. If any of the following levels are above average, you might need more tests:

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



HIV TEST

Introduction:

The HIV TRI-DOT test is a visual, rapid, sensitive and accurate immunoassay for the differential detection of HIV-1 & HIV-2 antibodies (IgG) in human serum or plasma using HIV-1 & HIV-2 Antigens immobilized on an immunofiltration membrane.

Procedure:

1. Add 3 drops of Buffer Solution to the centre of the device.
2. Hold the dropper vertically and add 1 drop of patient's sample 50µl (serum or plasma) using the sample dropper provided (use a separate sample dropper for each specimen to be tested).
3. Add 5 drops of Buffer Solution.
4. Add 2 drops of Protein-A Conjugate directly from the conjugate vial.
5. Add 5 drops of Buffer Solution and read results.
6. Read results immediately and discard the device considering it to be potentially infectious

INTERPRETATION OF RESULTS : NON-REACTIVE:

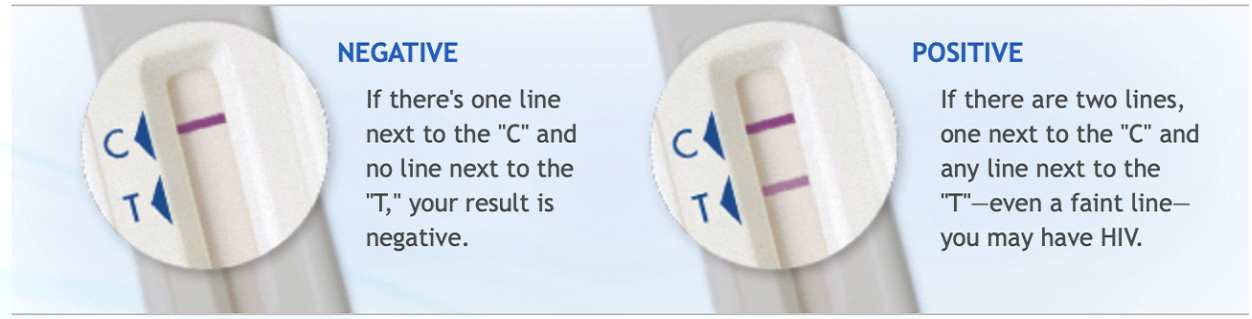
1. If only one Dot (only the Control Dot) appears the specimen is non reactive for antibodies either to HIV-1 or HIV-2. Interpret sample as non-reactive.

REACTIVE:

1. If two Dots, one for the control and the other for HIV-1 appear the specimen is reactive for antibodies to HIV-1.
2. If two Dots, one for the control and the other for HIV-2 appear the specimen is reactive for antibodies to HIV-2.
3. If all the three Dots, one each for control, HIV-1 & HIV-2 appear the specimen is reactive for antibodies to HIV-1 & HIV-2.

INVALID TEST:

If no Dot appears after the test is complete, either with clear background or with complete pinkish/purple background the test indicates ERROR. This may indicate a procedural error or deterioration of specimen/reagents or particulate matter in the specimen. The specimen should be tested on a new device.



BLOOD GROUPING

Introduction:

ABO blood group system, the classification of human blood based on the inherited properties of red blood cells (erythrocytes) as determined by the presence or absence of the antigens A and B, which are carried on the surface of the red cells. Persons may thus have type A, type B, type O, or type AB blood. The A, B, and O blood groups were first identified by Austrian immunologist Karl Landsteiner in 1901.

Your blood group is determined by the genes you inherit from your parents. Each group can be either Rh positive or Rh negative, which means in total there are eight main blood groups. Landsteiner found that there are substances in the blood, antigens and antibodies, that induce clumping of red cells when red cells of one type are added to those of second type.

Principle:

The ABO and Rh blood grouping system is based on agglutination reaction. When red blood cells carrying one or both the antigens are exposed to the corresponding antibodies they interact with each other to form visible agglutination or clumping.

Requirements:

1. Slides

2. Mixing stick
3. Blood sample
4. Antisera: anti-a, anti-b, anti-d

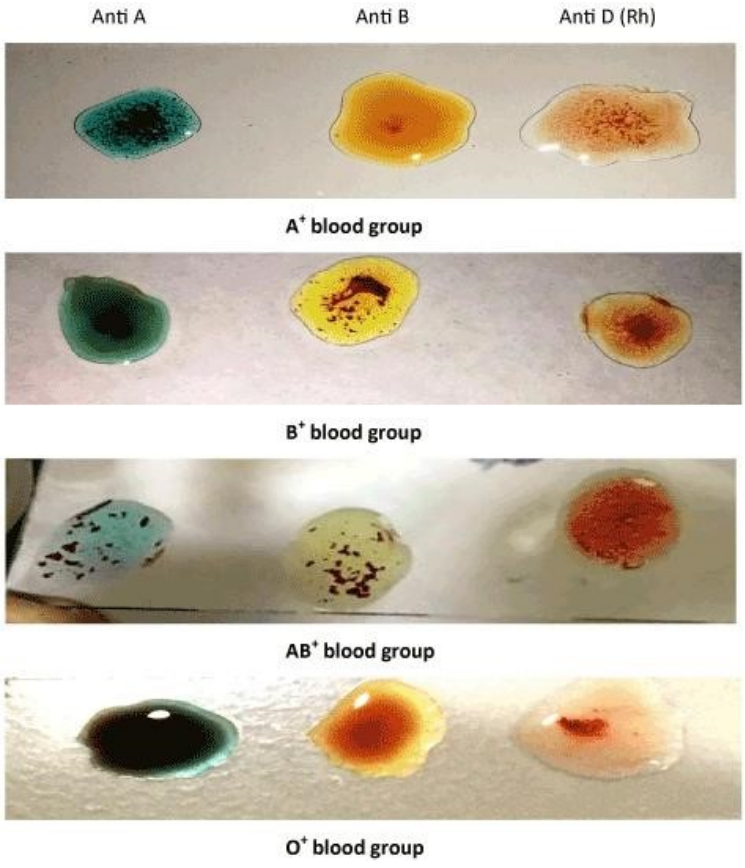
Procedure:

1. Take 3 clean slides and label them a,b and d.
2. Place a drop of blood sample in each of the slide.
3. Add equal amount of anti a, anti b and anti d antisera to each drop of blood and mix thoroughly by using mixing stick.
4. The slides are gently rocked for two minutes and results are observed.

Results:

- If agglutination is observed when individual's blood is mixed with Anti A reagent, then the individual is said to have a blood group "**A**".
- If agglutination is observed when individual's blood is mixed with Anti B reagent, then the individual is said to have a blood group "**B**"
- If agglutination is observed when individual's blood is mixed with Anti A and Anti B reagent, then the individual is said to have a blood group "**AB**"

- If no agglutination is observed when individual's blood is mixed with Anti A and Anti B reagent, then the individual is said to have a blood group "**O**"
- If agglutination is observed when individual's blood is mixed with Anti RhD reagent, then the individual is said to have a **"+ve**" Rh factor.
- If no agglutination is observed when individual's blood is mixed with Anti RhD reagent, then the individual is said to a have **"-ve**" Rh factor.



BLOOD GLUCOSE TEST

Introduction:

A blood glucose test measures the glucose levels in your blood. Glucose is a type of sugar. It is your body's main source of energy. A hormone called insulin helps move glucose from your bloodstream into your cells.

Too much or too little glucose in the blood can be a sign of a serious medical condition. High blood glucose levels (hyperglycemia) may be a sign of diabetes, a disorder that can cause serious, long-term health conditions.

Low blood glucose levels (hyperglycemia) are common among people with type 1 diabetes and people with type 2 diabetes who take certain diabetes medicines. Certain conditions, such as liver disease, may cause low levels of blood glucose in people without diabetes, but this is uncommon. Without treatment, severe low blood sugar can lead to major health problems, including seizures and brain damage.

Principle:

Fasting blood sugar and postprandial blood sugar measures the blood glucose at a specific time. Normal range of FBS is 70-110 mg/dL and of PPBS is <140 mg/dL. The enzyme glucose oxidase reacts with glucose, water and oxygen to form gluconic acid and hydrogen peroxide. The hydrogen peroxide can then be used to oxidize a chromogen or the consumption of oxygen measured to estimate the amount of glucose present.

Requirements:

1. Cuvette
2. Elastic band
3. Needle
4. Antiseptic
5. Reagent kit
6. Biochemical analyser

Procedure:

1. Area is cleaned with antiseptic.
2. Elastic band is wrapped around upper arm, causing veins to swell with blood.
3. Sterile needle is inserted to vein.
4. Blood is then drawn into tube attached to the needle.
5. Blood sample is added to vaccuatiner.
6. Later on blood sample is transferred to sterile cuvette.
7. After adding reagent kit, cuvette blood sample is placed in biochemistry analyser which displays the result.

Result:

- A normal blood glucose level is lower than 140 mg/dL (7.8 mmol/L).

- A blood glucose level between 140 and 199 mg/dL (7.8 and 11 mmol/L) is considered impaired glucose tolerance, or prediabetes. If you have prediabetes, you're at risk of eventually developing type 2 diabetes. You're also at risk of developing heart disease, even if you don't develop diabetes.
- A blood glucose level of 200 mg/dL (11.1 mmol/L) or higher may indicate diabetes

BLOOD GLUCOSE LEVELS CHART

LEVEL	mg/dl	mmol/L	RISK	SUGGESTED ACTION
DANGER - HIGH	315+	17.4	VERY HIGH	MEDICAL ATTENTION
HIGH	280	15.6	HIGH	MEDICAL ATTENTION
HIGH	250	13.7	HIGH	MEDICAL ATTENTION
HIGH	215	11	HIGH	MEDICAL ATTENTION
BORDERLINE	180	10	MEDIUM	CONSULT DOCTOR
BORDERLINE	150	8.2	MEDIUM	CONSULT DOCTOR
BORDERLINE	120	7	MEDIUM	CONSULT DOCTOR
NORMAL	108	6	NO RISK	NO ACTION NEEDED
NORMAL	72	4	NO RISK	NO ACTION NEEDED
LOW	70	3.9	MEDIUM	CONSULT DOCTOR
DANGER - LOW	50	2.8	HIGH	MEDICAL ATTENTION

Skills learned and the Experiences

Hands-on lab skills:

Developing safety skills

Record keeping

Manipulating glassware

Weighing

Purifying materials

Conducting distillations

Running columns

Using instrumentation

Building instrumentation

Troubleshooting

Data entry

Phlebotomy

Conclusion

From my internship at Dr. Bhatt Laboratory, I was able to get a better understanding of how the laboratory techniques work and how effective it is. I enjoyed working with the team to devise and implement different techniques. But, I still have a long way to go in understanding the different aspects of working skills, and I require to build up my accuracy skills as well.

Overall, I found the laboratory internship experience to be positive, and I am sure I would be able to use the skills I learned in my career later.

During work in this laboratory we experienced that how to work with doctors, lab technicians and staff.

Also learned that how to operate some automated machines, how to work with this machine, how to communicate with patients.

Future Aspects

The Future aspects after completing an internship coarse with Dr. Bhatt Laboratory is to establish mastering technical skills, knowledge on different applications, improving Interpersonal skills by challenging ourselves to perform various tests and trying to be 100% accurate on the skills for avoiding the least mistakes possible. To serve the best to every patient approaching at our door step.

References

- Laboratory Manual for Technicians
- Kawthalkar Essentials of Haematology
- Practical manual of medical Diagnostics
- Wikipedia