

## **EXPERIMENT-1**

### **MEASUREMENT OF BLOOD PRESSURE MANUALLY USING A SPHYGMOMANOMETER**

#### **AIM OF THE EXPERIMENT:-**

To learn the measurement of arterial blood pressure using a sphygmomanometer and to compare the results against a digital blood pressure measuring device.

#### **OBJECTIVES:-**

1. To measure the systolic blood pressure (SBP) and diastolic blood pressure (DBP) by percussions and auscultation.
2. To derive pulse pressure (pp) and main arterial pressure (MAP).
3. To categorise the results as normal blood pressure or mild, moderate and severe high blood pressure (hypertension)
4. To measure the blood pressure using a digital BP device and compare with that of mutual method.

#### **REQUIREMENTS:-**

1. SPHYGMOMANOMETER
2. DIGITAL BP MEASURING DEVICE
3. HEALTHY SUBJECT

#### **PRINCIPLES:-** 1. What is blood pressure and what is the derive blood pressure ?

2. The principles behind measuring systolic BP (SBP) and diastolic BP (DBP).

#### **BLOOD PRESSURE / ARTERIAL BLOOD PRESSURE :-**

- The arterial BP is the lateral pressure exerted by the flow against the wall of the artery . This is pulsatile in nature.
- The BP rises between the highest point (sbp) and lowest point (dbp) as per the cardiac cycle.
- The normal range of sbp should be between 90-120 mm of hg.
- The normal range of dbp should be between 60-90 mm of hg.

#### **PULSE PRESSURE :- THE DIFFERENCE BETWEEN (SBP-DBP) MM OF HG**

**MAIN ARTERIAL PRESSURE :-** This indicate the avg. BP of the arteries in a cardiac cycle  $MAP = 2/3 DBP + 1/3 SBP = 1/3 (SBP-DBP)+DBP$

#### **HOW IS BLOOD PRESSURE MEASURED :-**

- Arterial BP is measured by the turbulence in the blood as a korotkoff sound.
- The BP flows inside the arteries . It is not turbulent hence ,there is no korotkoff sound.
- During the 1st phase of BP measurement the pressure around the artery is measured by inflating the cuff of the sphygmomanometer which is usually measured from the brachial artery.

-This korotkoff sound reaches a peak then it decreases till it disappear when the ext. Pressure completely abstracts the artery i.e. it is equal to the highest arterial BP. The inflation should be continued beyond this point 180-200.

-Gradually deflates the cuff , when the korotkoff sound reappears indicates lowest arterial wall pressure aka DBP.

**ABNORMAL BP** :- It usually marked by the DBP.

The DBP is usually  $\leq$  SBP .

DBP $\leq$  60 SBP  $\leq$ 90 DBP $>$ 90 $\leq$ 100 IS MILD HYPERTENSION.

DBP  $>$ 110 IS SEVERE HYPERTENSION

DBP $>$ 120 IS EMERGENCY HYPERTENSION/HYPERTENSION CRISIS.

100 $<$ DBP $\leq$ 110 MODERATE HYPERTENSION

-Condition called isolated systolic hypertension common in elderly people, also common in adults who suffer from anaemia.

**WORKING OF DIGITAL BLOOD PRESSURE DEVICE** :- It was a pixo electric device or sensor which vibrate korotkoff sound using inflation. So ,that an output current is obtained. When the pressure reaches SBP ,the korotkoff sound disappears and becomes zero. This device is programmed to inflect beyond this point then start deflecting which is systolic BP.

**PROCEDURE:-**

1.a proper sized blood pressure cuff was used to begin BP measurement.

2. the length of the cuff bladder should be at least equal to 80 % of the circumference of the upper arm.

3.the cuff was wrapped around the upper arm with the cuff's lower edge, one inch above the antecubital fossa.

4. the stethoscope bell was pressed lightly over the brachial artery just below the cuffs edge.

5.rapidly the cuff was inflated to 180 mm hg . The air was released from the cuff at a moderate rate of 3 mm/sec.

6.it was listened with stethoscope and simultaneously observed by sphygmomanometer.

7.the systolic BP was observed by it's 1st knocking sound (korotkoff).the diastolic pressure was observed ,when the knocking sound would disappear.

8.the pressure of both arms recorded and the difference was noted.

9.the subject position, which arm was used, and the cuff size was recorded.

10. Measuring of BP was done two additional time ,wasting a few minutes between measurements, if the subjects pressure was being elevated.

SL NO	SUBJECT CODE	AGE	SBP	DBP	PP	CATEGORY
1	MS-L001	18	132	68	60	Pre hypertension
2	MS-L002	20	120	68	74	Normal
3	MS-L003	43	106	84	86	Normal
4	MS-L004	39	104	60	54	Normal
5	MS-L005	60	122	80	90	Pre hypertension
6	MS-L006	18	122	62	80	Pre hypertension
7	MS-L007	57	126	78	66	Pre hypertension
8	MS-L008	27	118	60	68	Normal
9	MS-L009	20	110	40	68	Normal
10	MS-L0010	18	95	56	56	Normal
11	MS-L0011	63	114	76	80	Normal
12	MS-L0012	20	128	78	60	Pre hypertension
13	MS-L0013	46	108	82	66	Normal
14	MS-L0014	29	126	76	64	Pre hypertension
15	MS-L0015	45	116	50	64	Normal
16	MS-L0016	40	138	84	46	Hypertension Stage 1
17	MS-L0017	48	110	70	68	Pre hypertension
18	MS-L0018	18	146	74	68	Pre hypertension
19	MS-L0019	50	112	86	68	Pre hypertension
20	MS-L0020	20	136	80	68	Pre hypertension
21	MS-L0021	47	122	68	62	Pre hypertension
22	MS-L0022	19	112	78	70	Normal
23	MS-L0023	55	100	78	72	Normal
24	MS-L0024	23	106	68	74	Normal
25	MS-L0025	21	134	80	64	Pre hypertension
26	MS-L0026	19	128	78	50	Pre hypertension
27	MS-L0027	64	108	68	64	Normal
28	MS-L0028	30	106	68	50	Normal
29	MS-L0029	43	111	68	70	Normal
30	MS-L0030	23	120	68	58	Normal
31	MS-L0031	64	140	62	60	Hypertension Stage 1
32	MS-L0032	40	98	56	88	Normal
33	MS-L0033	23	126	68	84	Pre hypertension
34	MS-L0034	44	154	86	76	Hypertension Stage 1
35	MS-L0035	50	130	74	46	Pre hypertension

	AGE	SBP	DBP	PULSE
MEAN	36.11	119.57	71.08	66.86
STANDARD DEVIATION(SD)	15082	13.91	10.60	11.21
Standard error of mean(SEM)	2.67	2.35	1.79	1.895

	Correlation between	
<u>1</u>	Age and BP	-0.1141303
<u>2</u>	BP and Pulse	-0.0907077

**RESULTS:-** The mean systolic and diastolic BP were – 119.57 +- 2.35 and 71.08 +- 1.79 respectively. Type correction found between age and BP was - - 0.1141303 and between BP and pulse was – 0.0907077

Out of 35 subjects , 19 were in normal BP range , 14 were in prehypertension range and 2 were in hypertension stage-1.

**PRECAUTION:-**

- BEFORE MEASURING THE BP USING SPHYGMOMANOMETER , MAKE SURE TO PLACE STETHOSCOPE OVER BRACHIAL ARTERY.
- USE A LARGER CUFF ON HEAVILY MUSCLED SUBJECT.
- DON'T PLACE THE CUFF OVER CLOTHING.
- ITEMS SHOULD BE SUPPORTED AND PLACED AT ABOUT HEART LEVEL.

**By:- SATWIK SRIMANSU SAHOO**

**ROLL NO. -120BM0014**

## **EXPERIMENT 2A**

### **Aim of the experiment:**

Estimation of cardiac electrical activity using ECG.

### **Requirements:**

1. A 12 lead ECG system (I121)
2. 9 electrodes
3. Electrode gel
4. Volunteers

### **Principle:**

What is ECG?

- ECG represents the vectorial electrical activity of the cardiac muscle.
- The ECG signal is quaxy periodic signal.
- The contraction of the cardiac muscle (conducting zone) is autorhythmic & synchronous.
- ECG measures the resultant electrical activity of cardiac axis.

ECG leads:

- In a 12 lead system uses 9 electrodes.

Leads are the anatomical functional positions of the electrodes.

Leads are two classes:

- Limb leads
- Chest leads
  - a. Limb leads: there are plane and augmented.
    - Lead I, lead II, lead III are plain limbs leads.
    - a VR, a VL, a VF are augmented leads which are derived leads.

For limb leads we need 3 electrodes.

- b. Pre cordial limbs: These are v1, v2, v3, v4, v5, v6. The locations of precordial limbs:
  - V1 is 2<sup>nd</sup> intercostal space right border of sternum.
  - V2 is 2<sup>nd</sup> intercostal space left border of sternum.
  - V3 is 4<sup>th</sup> intercostal space right border of sternum.
  - V4 is midpoint between v3 & v5.
  - V5 is 5<sup>th</sup> intercostal space along the mid clavicular line.
  - V6 is 5<sup>th</sup> intercostal space along anterior axillary line.

For precordial limbs we need 6 electrodes.

The augmented limb leads are derived by comparing one limb electrode with the average of other two electrodes.

How can you be sure that leads are properly placed?

→ Leads I,II,III are the potential differences between the limbs as indicated.

RA & LA are the right and left arms and LL is the left leg.

$$\text{II} - \text{I} - \text{III} = 0 \dots\dots\dots \text{Eqn (i)}$$

$$\text{II} = \text{I} + \text{III} \dots \text{Eqn (ii)}$$

$$I = II - III \dots \text{Eqn (iii)}$$

$$\text{III} = \text{II} - \text{I} \dots \text{Eqn (iv)}$$

To check that the leads are properly placed see that whether they satisfy eqns or not.

→ Axis of heart is imaginary and it is variable.

→ The normal axis of the heart lies between -30 to +60.

→ If it goes to the left it means the heart is more to the left side and it is called left ventricle hypertrophy.

If it goes to the right it means the heart is more towards the right side & it is called right ventricle hypertrophy.

Witson's central terminal represents the average of the limb potentials.

## Waveforms:

P.wave: reflects atrial depolarization (activation)

PR interval: It's the distance between the onset of the P-wave to the onset of the QRS complex.

PR segment: It represents the depolarization of the ventricles. QRS duration is the time interval from the onset to the end of the QRS complex.

**QRS complex:** It represents the depolarization of the ventricles. QRS duration is the time interval from the onset to the end of the QRS complex.

T wave reflects the rapid polarization of contractile cells and T wave changes occur in a wide range of condition.

U wave is seen occasionally. It is a positive wave occurring after the T wave.

Individuals with prominent T waves, as well those with slow heart rates, display U waves more often.

## Rule of four:

### Rate axis

## Rhythm- Wave forms

## **Rate**

How to calculate rate:

- Rule of 300 (or 1500) divide 300 (or 1500) by the numbers of large (or small) boxes between each QRS is rate.
- In ECG graph, y-axis represents voltage & x axis represents timeline (in seconds).

Tachycardia is the condition when the no. of beats is more than 100 per minutes and the rhythm is regular. When it becomes irregular, it is Tachyarrhythmia.

$HR > 100 = \text{Tachycardia}$

HR of 60-100 per minute is normal.

Bradycardia is the condition when the no. of beats is less than 60 per minute.

$HR < 60 = \text{Bradycardia.}$

## **Rhythm:**

First identity of rhythm originates from sinoatrial node. It is a point in right atrium from where the heart beat originates.

## **Normal sinus rhythm:**

- Rhythm should be regular
- P-wave should be in the direction to that of QRS complex.
- ECG can be divided into narrow complex & wide complex on the QRS complex direction.

QRS complex direction = 0.08 to 0.10 seconds is normal

QRS complex duration = 0.10 to 0.12 seconds, it is intermediate

## **Normal intervals:**

The wave form is analysed by interpreting 3 peaks (P, QRS, T) and 3 intervals/segment.

PR interval = 0.20 sec.

During estimation of the R peaks by various algorithms, a blanking interval of equivalent to PR interval to PR interval is always taken.

## **Prolonged QT:**

- Direct QT interval is not a reliable marker because QT interval is dependent upon body surface area indirectly.
- It varies between male & female: For normal, Men – 450 ms, Women – 460 ms.

$$\text{Corrected QT (QTC)} = \text{QT}_{\text{M}} / \sqrt{(\text{R}-\text{R})}$$

(R - R) indicates one full cardiac cycle or one heartbeat.

Standard drum speed = 25 mm/sec.

We can increase or decrease the drum speed.

Statistical indices & geometrical indices.

Poin care plots: Qualitative analysis.

SD1 represents the dispersion of the point perpendicular to the line of identity.

SD1 width represents the instantaneous change in the heart variability (parasympathetic activity) or effect on the heart.

SD2 represents the dispersion of the point along the line of identity (sympathetic effect on heart)

SD1: SD2 ratio represents the most significant clinical indicator of autonomic activity of the heart.

$$\text{SD1}^2 = \frac{1}{2} (\text{SDSD})^2$$

$$\text{SD2}^2 = 2 (\text{SDRR})^2 - \frac{1}{2} (\text{SDSD})^2$$

The RMSSD, the SDNN shows equivalent outcome with SD1 & SD2 respectively.

In case of premature (HR is 160) and new born (HR is 120).

If intervals are low and HR is fast, they are healthy but if intervals are high, heart rate is slow; this situation is for an all patient in ICU.

In 25 years old (SD1 & SD2 is the most). So, the HRV is more and parasympathetic activity is more.

Parasympathetic: decrease the heart rate & increases instantaneous variability of heart.

Sympathetic: increase the heart rate.

The LF represents parasympathetic activity with vagal modulation. The HF represents sympathetic activity.

Heart rate decreases and sympathetic activity decreases while sleeping.

$$\text{VLF} = \text{Very Low Frequency}$$

Athlete have resting HR close to 40 bpm.

Normal adults have between 60-100 bpm.

If your body is losing sodium your BP will fall.

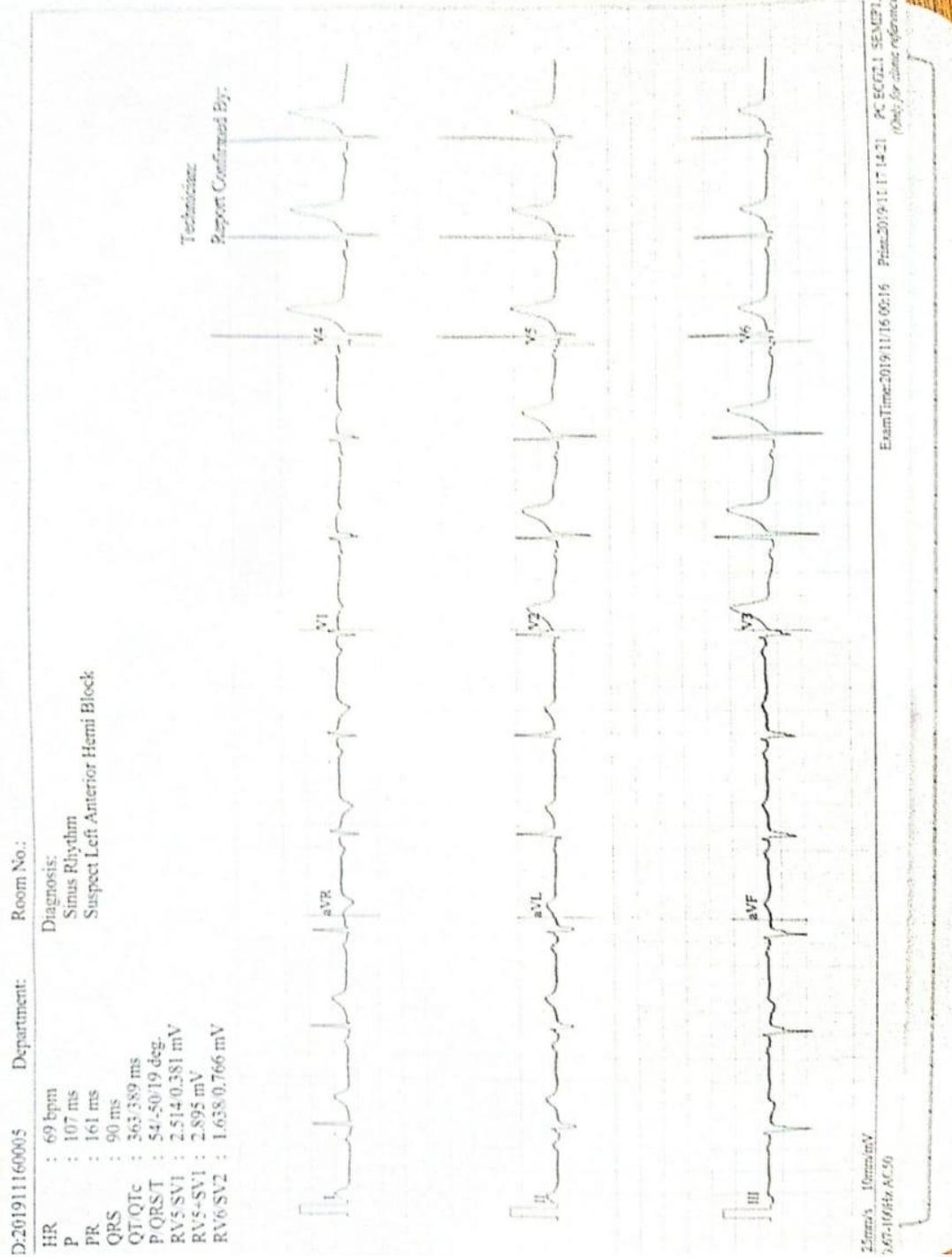
## **Procedure:**

Recording a normal ECG:

1. Each student should record the ECG of one of the members of the lab group and have their own ECG record.
2. The subject should be relaxed all the time.
3. Attach the four limb leads to the approximate limb as marked on each lead (RA: right arm, LL: left leg etc.)
4. Also attach the six chest leads using the electrode pads and approximately marked leads.
5. It is important that the subject remain relaxed while the ECG is recorded.
6. Record a few centimeters (about 4 or 5 complexes) of each the 3 standard limb leads (I,II,III), and the three augmented leads (aVF, aVR, aVL).
7. At the beginning and end of the recording for each person, set the recorder in the standadixation position (STO) and the depress the standardixation position.
8. The standard is 1 mv and should yield a deflection of 10 nm at a recording sensitivity of 1.
9. Record the unipolar chest leads. The position for the chest leads should be studied well
10. Mount your own ECG in the folder provided.
11. Also calculate the mean electrical axis of the heart. Be sure to get the sum of all the positive and negative deflection to determine the total voltage in each lead.
12. A positive deflection is measured from the top of the isoelectric sine to the peak of the deflection.
13. A negative deflection is measured from the bottom of the isoelectric line to the peak of the deflection.
14. Plot the potential obtained from lead I and II on the graph.
15. Calculate the mean electrical axis.

Record the value on the mean electrical axis on the folder.

## R&RM LAB, NIT ROURKELA ECG Report



### Results:

The duration of one cardiac cycle (the R-R interval) was found as:

$$\text{R-R interval (big boxes)} = 4$$

$$\text{R-R interval (mm)} = 20 \text{ mm}$$

Paper speed (standard) = 2.5 mm/s

By using:

$$\begin{aligned}\text{Heart rate (bpm)} &= 1500 / (\text{R} - \text{R}_{\text{mm}}) = 1500 / 20 \\ &= 75 \text{ beats per minute (bpm)}\end{aligned}$$

Indicating normal HR, since normal range is (60-100) bpm.

Indicating normal sinus rhythm (PR- interval remained constant)

On the other rule of four criteria axis, indicates normal since,

For normal P-klave axis: P-klaves should be upright in leads (i) & (ii) as well as inverted in aVR as is in our report.

Wave forms, indicates normal in our R and RM lab report:

- PR segments- is less than one big box (approx.: 0.20 sec)
- QRS segment- is less than two small boxes (approx.: 0.08 sec)
- QT interval: covers 2 large boxes.  
$$\begin{aligned}5 \text{ mm (large box)} &= 0.2 \text{ sec (200 ms)} \\ &= (2 \times 200) \text{ milliseconds.} \\ &= 400 \text{ ms or } 0.4 \text{ sec (normal)}\end{aligned}$$

### **Conclusion:**

we learnt anatomical position of 12-ECG leads system on a subject and interpreting of it's results. Our subjects HR was 75 bpm, PR segment 0.20 sec, QRS interval of 0.08 sec and QT interval 400 ms, all readings are normal.

### **Precaution:**

1. Patient skin should be dry, hairless and oil free. Shave hair that can possibly impede electrode placement.
2. Promote an environment that prevents the patient from sweating.
3. With arms lying flat on the side, ask the patient to relax the shoulders and keep the legs uncrossed.

Place patient/ subject in spine position or elevated bed.

**By:- SATWIK SRIMANSU SAHOO**

**ROLL NO. -120BM0014**



# **EXPERIMENT-2B**

## **AIM OF THE EXPERIMENT:**

To learn how to use HRV Software.

## **THEORY:**

HRV: Heart Rate Variability

It is simply a measure of the variation in time between each heartbeat. This variation is controlled by a primitive part of the nervous system called the automatic nervous system (ANS).

HRV is an interesting and non-invasive way to identify the ANS imbalance. If a person's system is in more of a fight or flight mode, the variation between subsequent heartbeats is low. If one is in a more relaxed state, the variation between beats is high.

### **Poincare Plots:**

It is a type of sequence plot used to quantify self-similarity in processes, usually periodic functions.

The Poincare plot analysis is a geometrical and non-linear method to access the dynamics of HRV.

### **FFT:**

Fast Fourier Transform (FFT) is an algorithm that computes the Discrete Fourier Transform (DFT) of a sequence or its inverse (IDFT). Fourier analysis converts a signal from its original domain to a representation in the frequency domain and vice versa.

**There are 3 types of frequencies: -**

1. VLF -very low frequency
2. LF - low frequency (0.04-0.15Hz)
3. HF – high frequency (0.15-0.21Hz)

## **STEPS IN HRV MEASUREMENT/PROCEDURE: -**

1. Noise elimination
2. Type || lead
3. To pressure R- peak

## **METHOD:** -

The raw ECG was first processed by using a band pass filter with cut-off frequencies of 0 and 16 Hz.

### **Description: -**

1. Atmosphere thermal noise
2. Power frequency noises.

## **DETECTION OF R- WAVE:**

### **1.Peak level estimation:**

2 mins duration rectified signal was sorted in decreasing order.

The first two data points (1<sup>st</sup> two highest peaks) were averaged to get an estimation of R-peak amplitude.

### **METHOD: - Recission Rule (Thresholding):**

70% of the average R-peak was taken as threshold to detect R-peak from the rest of the signal.

A blanking interval of > 200ms is taken in sample reach procedure.

## **MRV CALCULATION: -**

### **Method: -**

1. Time Domain (Geometrical)
2. Frequency Domain (FFT)

Connect the ECG leads for lead-2. Attach finger pulse.

Check computed input for Heart Rate (HR).

### **Learn how to verify.**

1. Have the subject still. Record baseline control issues.

Have subject take a deep breath and hold than push against a cold glottis. Record and make a comment. (e.g.: -Valsalva maneuver). In the zoom windows observe overlay & layered looks.

## **OBTAINED TIME-DOMAIN PARAMETER FOR HRV ANALYSIS (SUBJECT-1)**

The HRV values are fed into the Kubios HRV software to analyse the data: -

SN	R-R Interval						
1	1216	13	1223	25	1197	37	1295
2	1324	14	1261	26	1307	38	1257
3	1234	15	1113	27	1178	39	1221
4	1228	16	1288	28	1277	40	1258
5	1204	17	1225	29	1319	41	1194
6	1221	18	1256	30	1273	42	1271
7	1275	19	1295	31	1247	43	1222
8	1197	20	1145	32	1232	44	1237
9	1285	21	1296	33	1308	45	1279
10	1225	22	1205	34	1230	46	1166
11	1263	23	1235	35	1325	47	1244
12	1216	24	1265	36	1260		

### OBTAINED TIME-DOMAIN PARAMETER FOR HRV ANALYSIS (SUBJECT-2)

SN	R-R Interval						
1	1010	15	1011	29	1034	43	1080
2	1058	16	1068	30	1051	44	1029
3	1008	17	1019	31	998	45	1059
4	1032	18	1019	32	1040	46	1109
5	1080	19	1068	33	1051	47	1021
6	1039	20	999	34	989	48	1058
7	1069	21	1031	35	1071	49	1071
8	1090	22	108	36	1040	50	1009
9	1008	23	970	37	1033	51	1-39
10	1040	24	1020	38	1070	52	1039
11	1031	25	999	39	1008	53	991
12	1003	26	1009	40	1047	54	1041
13	1062	27	1063	41	10299	55	1038
14	1029	28	1044	42	1020	56	1020

### OBTAINED TIME-DOMAIN PARAMETER FOR HRV ANALYSIS (SUBJECT-3)

SN	R-R Interval						
1	769	22	751	43	688	64	790
2	724	23	756	44	663	65	765
3	723	24	737	45	669	66	746
4	724	25	749	46	702	67	752
5	691	26	746	47	746	68	761
6	717	27	746	48	761	69	728
7	719	28	745	49	777	70	724
8	757	29	742	50	770	71	750
9	780	30	747	51	769	72	737
10	802	31	711	52	759	73	748
11	834	32	711	53	769	74	749
12	815	33	705	54	757	75	755
13	802	34	711	55	739	76	738
14	813	35	720	56	759	77	721
15	774	36	736	57	759	78	715
16	746	37	755	58	759	79	735
17	746	38	786	59	749	80	751
18	762	39	765	60	749	81	804
19	758	40	748	61	790		
20	741	41	727	62	765		
21	747	42	717	63	746		

## RESULTS AND OBSERVATIONS:

Table-1 Time Domain Analysis-

Time Domain Parameters	Subject-1	Subject-2	Subject-3	Healthy Control
Mean RR	1245 (ms)	1036 (ms)	747 (ms)	86.1 (ms)
SDNN(STDRR)	43.8 (ms)	26.7 (ms)	27.7 (ms)	99.0 (ms)
Mean HR	48 (bpm)	58 (bpm)	80 (bpm)	70.32 (bpm)
RMSD	76.8 (ms)	76.8 (ms)	20 (ms)	54.9 (ms)
NN50	24	16	1	519
PNN50	52.17%	29.09%	1.30%	28.1%

Table-2 Frequency Domain Analysis-

Frequency Domain Parameters	Subject-1	Subject-2	Subject-3	Healthy Control
VLF	15 ( $\text{ms}^2$ )	7 ( $\text{ms}^2$ )	40 ( $\text{ms}^2$ )	51.12 ( $\text{ms}^2$ )
LF	231 ( $\text{ms}^2$ )	146 ( $\text{ms}^2$ )	626 ( $\text{ms}^2$ )	14.88 ( $\text{ms}^2$ )
HF	1467 ( $\text{ms}^2$ )	397 ( $\text{ms}^2$ )	117 ( $\text{ms}^2$ )	33.30 ( $\text{ms}^2$ )
Total	1713 ( $\text{ms}^2$ )	549 ( $\text{ms}^2$ )	783 ( $\text{ms}^2$ )	99.30 ( $\text{ms}^2$ )
LF/HF	0.157	0.367	5.361	0.45

Table-3 Non-Linear Analysis-

Non-Linear Parameters	Subject-1	Subject-2	Subject-3	Healthy Control
SD1 (ms)	54.9	30.4	14.2	38.9
SD2 (ms)	28.9	22.3	36.3	134.4

## OBSERVATIONS:

- Low values of SDNN indicate no variability and it is possibly the most used index to measure the long term HRV.

### FROM TABLE-1

1. Subjects 2 and 3 have way too low SDNN value compared to the healthy control value.
2. Indicating less variability of their HRV.
3. Indicating possibility of coronary artery disease (ARTERIES NARROW DOWN AND BUILT-UP FATTY PLAQUES)
4. Thus, decrease in blood flow to certain heart parts.

### **FROM TABLE-2**

1. LF/HF, value (ratio).
2. Subject 3 has a much higher LF/HF ratio.
3. Indicating increase in sympathetic activity and decrease in parasympathetic activity.
4. But for the healthy control/individual is stays close or at  $\text{LF}/\text{HF} = 0.45$

### **FROM TABLE-3**

Shows the short-term variability and long-term variability of all subjects and healthy individual.

### **CONCLUSION:**

1. In this experiment, we learnt how to use the KUBIOS HRV STANDARD SOFTWARE.
2. Further learnt that, heart rate variability is an important tool in cardiology, since it helps investigate the sympathetic and parasympathetic functions of the AUTOMATIC NERVOUS SYSTEM.

**By:- SATWIK SRIMANSU SAHOO**

**ROLL NO. -120BM0014**

# **EXPERIMENT-3**

## **AIM OF THE EXPERIMENT:-**

To identify major blood cells like RBC and 3 main categories of WBC (granulocytes, lymphocytes, and monocytes)

## **OBJECTIVES:-**

- 1) To identify the normal RBC or any abnormal RBC like microcytes, macrocytes, spherocytes.
- 2) To identify individual WBC : neutrophil, eosinophil, basophil, lymphocytes and monocytes.
- 3) To identify or separate different platelets.

## **REQUIREMENTS:-**

- 1) WHOLE BLOOD SAMPLE (UNCOAGULATED)
- 2) HISTOLOGICAL GLASS SLIDE
- 3) COVERSLEIPS
- 4) STAIN (HAEMATOXYLIN & EOSINE)
- 5) MICROSCOPE (LIGHT BINOCULAR OLYMPUS I35)
- 6) HAEMOCYTOMETER CHAMBER
- 7) RBC PIPETTE
- 8) RBC DILUTING FLUID

## **PRINCIPLES:-**

### **HOW WILL YOU IDENTIFY RBC ?**

They appear as biconcave discs of uniform shape and size. The size is around 7.5 to 8.5 um. RBC lack organelles and granules. RBC have characteristic pink appearance due to their high content of haemoglobin. The central pole of each RBC is due to the concavity of the disc.

### **ABNORMAL RBCs:-**

Microcytes are the cells that are smaller than the normal macrocytes. All the cells that are larger than normal. Spherocytes are the cells which are sphere shaped rather than biconcave disc shaped or normal.

### **HOW WILL YOU IDENTIFY WBCs ?**

WBCs are the round uniform nucleus and have small amount of cytoplasm surrounding it. The size is around 12.17 um. They are about 3 times the size of RBC.

### **TYPES OF WBCs:-** Granulocytes-These are a types of WBC that has protein containing granules.

These are of 3 types – 1) Neutrophiles

- 2) eosinophiles
- 3) basophiles

### **NEUTROPHILES:-**

These are the highest no. Of WBCs (40-60%). Cytoplasm is highly granular. Nucleus is multilobed. Neutrophiles are major scavenger cells which acts as first line of defence.

**EOSINOPHILES**:- These are the next major of granules after neutrophiles(4-6%). They have bilobed nucleus. Their major granule is hystamine. Eosinophiles contains acidic granules which is pink in appearance. Eosinophiles are disease fighting white blood cells.

**BASOPHILES**:- These are only 1 % granules are larger. Nucleus is large and occupies majority of cytoplasm. Basophiles can be easily stained by basic stain.

**LYMPHOCYTES**:- These are 20 – 40 %.They play an important role in your immune system, helping your body fight off infection. Lymphocytes include natural killer cells, T cells and B cells.

**MONOCYTES**:- They have no granules. These are the largest type of leucocyte with very large kidney shaped nucleus. Have blue covered cytoplasm .Monocytes influence the process of adaptive immunity. These are 1.5%.

#### **PROCEDURE:-**

1.Blood should be carefully drawn to the 0.5 mark of the RBC pipette and excess blood clinging to the exterior of the pipette is removed by wiping it with a piece of cloth or tissue.

2.An isotonic solution should be drawn to the 101 mark to dilute the blood.

3.The blood and diluting fluid are mixed by shaking the pipette vigorously in a horizontal position for 2,3 minutes.

4.Several drops of mixed fluid are discarded and the end of the pipette is dried with a piece of lint free absorbant material.

5.The tip of the pipette is touched to the side of the hemocytometer chamber and a drop of a fluid will seen under the cover glass.

6.Wait for about 2,3 minutes as erythrocyte require settling time to assume a single level.

7.Total no. Of cells in 5 squares in the centre of counting chambers is determined under the high dry objective of the microscope.

8.To avoid duplicate countinmg of a single cell , you must count only those cell that touch the lower and left boundaries.

#### **CALCULATION:-**

RBC/ $\mu$ l or mm $^3$  = no. Of cell in 5 squares (80 small square) \*dilution no. \* reciprocal of volution

Dilution no. = 0.5 : 100 = 200

Each square contains 16 small squares and each small square has an area of 1/400 mm $^3$  and depth of 1/10 mm. A total of 80 squares are include in the counting.

$1/10 \times 1/400 \times 80 = 1/50$

RBC/ $\mu$ l = no. Of 200 \* 50

RBC/ $\mu$ l = no. \* 10,000

Normal RBC range in human:-

Male = 4.7 to 6.1 million per  $\mu$ l

Female = 4.2 to 5.4 million cells per  $\mu$ l

-Low RBC count (erythropenia) could indicate anaemia ,vit-B deficiency, internal bleeding ,kidney disease , malnutrition etc..

-High RBC count (erythrocytosis) could indicate cigarette smoking, problem with heart str. And functions,dehydration,kidney tumour , low blood oxygen level etc..

**PLATELET COUNT:-** For counting of total platelet cells, the same procedure and formula applies as that of RBCs. The only difference lies in the diluting solution. Diluting solution for platelet counting:- 1% Ammonium oxalate prepared by- (1 g Ammonium oxalate in 100 ml of distilled water) Normal platelet count :- Range =140,000 –450,000 cells per ul

**Low platelet count/Thrombocytopenia:-** Caused by :- Leukemia and certain other cancerous cells , certain types of anaemia , certain viral infection ,certain respiratory disorders , radio and chemotherapy, certain autoimmune disorders , iron and folate deficiency , vit-B12 deficiency etc..

**High platelet count :-** Maybe caused by : Bleeding ,cancer, certain infection, removal of spleen, certain type of anaemia, certain inflammatory disease , surgery etc..

**Conclusion:-** We learned to count RBCs and platelets from this experiment. We also learned types of RBCs and their abnormalities.

**PRECAUTIONS:-**

- 1)Counting chambers and pipettes should be clean and dry .
- 2)Fingertip and pricking lancet should be sterile.
- 3)Blood should be freely come out without squeezing.
- 4) Be careful to prevent clotting of blood inside the pipette.
- 5)Blood should be properly mixed with the diluting fluid.
- 6) Cell should be settled down and more or less evenly distributed before counting.

**By:- SATWIK SRIMANSU SAHOO**

**ROLL NO. -120BM0014**

## **EXPERIMENT - 4**

### **AIM OF THE EXPERIMENT:**

To learn the making of equine blood smear manually and review the peripheral blood smear under a microscope.

### **REQUIREMENTS:**

1. Fresh blood sample after venipuncture fingertip puncture.
2. An adequate number of medical and coverslips.
3. blood slides.
4. A camco quick stain (buffered).
5. Microscope
6. Chart
7. Distilled Water
8. Dropper
9. Petri Dish
10. Immersion Oil
11. Cotton

### **PRINCIPLE**

1. PBS or a Peripheral Blood Smear helps to determine the relative number of each type of WBC present in the Blood. Apart from this, a study of RBCs, WBCs, and Platelet morphology are also performed.
2. Wright's stain is a combination of Polychrome Methylene Blue and Eosin. It has a selective staining property that helps to identify different types of WBCs.
3. PBS also helps to quantitative different types of abnormal cells if present.

### **Types of Blood Smear:**

1. Thin Blood Smear: It is mainly useful for the detection of cell morphology and differential cell count.
2. Thick Blood Smear: It is most useful for detecting blood parasites especially malaria because they examine a larger sample of blood even if a few members of parasites are available in the blood at the time of the test additionally the percentage of RBC that are infected and what type of parasite is present can be detected.

## **Different types of stain in current use:**

1. Giemsa's Solution: It is used in insider genetics and for histopathological diagnosis of malaria. Giemsa's solution is a mixture of methylene blue, Eosin, and Azure B.
2. Wright- Giemsa Stain: It is a combination of Wright and Giemsa Stain.
3. May- Grinwald Stain: it produces a more intense coloration also takes a longer time to perform May-Grinwald and Giemsa's are stable for five years. The bottles must be kept closed the advice tourist temperature is 18 to 30 degrees Celsius.
4. Leishman Stain: it provides a clenched in quantity it is generally used to differentiate and identify leukocytes malaria parasites and Trypanosoma.

## **PROCEDURE: -**

### **Preparation of slide: -**

1. Place a drop of blood sample in the center of a labeled slide near its frustrated end (not at the edge)
2. Hold another slide at 45-degree angle to the drop. Align the drop to spread along the contact line of two slides
3. Then quickly push the upper slide to the frustrated end of the lowest slide
4. The smear has a good tail. This can be achieved by homogeneous spreading of the blood.
5. Allow the thin smear to dry and fix the smear by dripping them in absolute ethanol/methanol

### **STAINING:**

1. Take camco quick stain, dip the slide for 10 seconds, take it out.
2. Dip the slide in distilled water for 20 sec for good stain.

### **SMEAR REVIEW & DC:**

1. Take the slide and observe under low magnification for an overall impression and general appearance.
2. Check for event distribution of WBCs.
3. Now observe the slide under high magnification after putting a coverslip with a droplet of immersion oil.

4. Now estimate the WBC count by recording the number of WBCs for high power.
  5. Take at least 10 fields to identify different types of WBCs and note the count.
  6. Now you can get total number of WBCs and percentage of WBCs.
  7. Identify immature granulocytes (very large nucleus), dysplastic granulocytes (cytoplasm ratio high), abnormal granulocytes.
  8. Observe abnormal shaped RBCs in blood.
  9. Grade the RBCs as per the degree of variability in their size and shape.
10. Observe-
- Polychromatophilia
  - tear drop RBCs
  - Spherocytes
  - Elliptocytes
  - Ovalocytes
  - Basophilic stippling
11. Observe the degree of color
- hyperchromia—bright red
  - hypochromia—light red

### RBCs Review

1. Most numerous of blood cells, 4-6 million/mm cube
2. Biconcave and disc shaped
3. Devoid of nucleus (mammals)
4. Rich in hemoglobin red color
5. Normal shaped RBCs are normocytic
6. Normal number of RBCs are normochromic

### Roleaux formation: -

1. Aggregates of RBCs in glutination, some antibiotics are formed against RBCs. This is a severe medical condition.
2. 3-4 RBCs – slight
3. 5-10 RBCs – moderate
4. 10+ RBCs – marked

Semiquantitative the abnormal cells-

0-2 few

3-6 moderate

6+ many (myelodysplastic condition, cancerous cells)

If the eosin is more than 10%

Basophils are more than 5%  
Blastocytes are more than 20%  
This is CANCEROUS CONDITION.

### **Platelet Review-**

1. The estimate is made by counting the avg. no. of platelet seen per 100 x (objective) in immersion field.
2. When the no. is multiplied by 15000 this gives you the no. of platelets per micro litre of blood.
3. 1.5- 3 Lac → adequate no of platelets.

### **Neutrophil-**

1. Most abundant WBCs (60-70).%
2. Size 12-15 micrometre
3. Nucleus 2.5 lobed, each nuclear lobe connected by chromatin.
4. Cytoplasm contains numerous fine granules.
5. Nucleus stains deep blue.
6. Phagocytic in nature.

### **Eosinophil-**

1. 4th most abundant WBCs (2-4%)
2. Size 12-17 mm
3. Nucleus usually bilobed.
4. Nucleus stains deep purple.
5. Nucleus not marked by granules.

### **Basophil -**

1. 5th and least abundant WBCs
2. Size 14-16 mm
3. Nucleus usually bilobed.
4. Numerous, large, cytoplasmic granules
5. Nucleus also stains deep purple.

### **Monocyte-**

1. 3rd most abundant WBC (3-8%).
2. Large sized WBCs - 15-18mm.
3. Generally, avoid with large usually reuniform nucleus.
4. A granulocyte.
5. Easily identified by their large size with purple stained nucleus

### **Lymphocyte-**

1. 2nd most abundant WBC.
2. Size - 6-9 mm (small) and 10-14 mm (large)
3. Nucleus occupies most of the cell volume
4. Nucleus stains deep purple, cytoplasm light purple.
5. small and large lymphocytes occur.

### **CONCLUSIONS**

We learned to prepare Equine blood smear and also PBS. We studied normalities and abnormalities of all.

### **PRECAUTIONS**

- 1) The smear should be prepared with utmost importance. It should be thin, monolayer.
- 2) If you are getting clumps of platelets while using EDTA blood, then discard the slide
- 3) Vortex the EDTA tube for 15 sec, the slide should be remade.
- 4) Immediately observe the smear under the microscope.
- 5) Be careful while preparing the slides. All the processes should be followed correctly to get a good slide.

**By:- SATWIK SRIMANSU SAHOO**

**ROLL NO. -120BM0014**

## **EXPERIMENT-5**

### **HAEMATOLOGY: CELL COUNT USING HAEMATOCYTOMETER**

#### **AIM OF THE EXPERIMENT:-**

To learn total lobe count (TLC) using haematocytometer.

#### **REQUIREMENTS:-**

- Fresh blood cells.
- Sodium/Potassium EDTA tube.
- WBC diluting solution.
- Haemocytometer.
- Microscope , Micro-pipette.

#### **PRINCIPLE**

Turk solution principle is that the solution lysis RBCs. It minimally damages WBCs and thus protective. The composition of turk solution, glycol acetic acid 2 ml , Gentian violet 1ml , sodium chloride 0.9g.

#### **PROCEDURE**

- 1.Blood should be carefully drawn to the 0.5 mark of the WBC -pipette and excess blood clinging to the exterior of pipette is removed by wiping it with a piece of cloth.
- 2.WBC reagent (Glacial acetic acid 3%) should be drawn to the 11 mark to dilute the blood .
- 3.The blood and the diluting fluid are mixed by shaking the pipette vigorously in a horizontal position for 2-3 minutes.
- 4.Several drops(2-4) drops of mixed fluid are disordered and the end of the pipette is dried with a piece of lint free absorbent material.
- 5.The tip of the pipette is touched to the side of the haemocytometer counting chamber and a drop of a fluid will run under the cover glass.
- 6.Wait for about 2-3 mins as leukocytes require setting time to assume a single level.
- 7.Total number of cells in 4 squares at the corners of counting chamber is determined under the low objective of the microscope.
- 8.To avoid duplicate counting of the single cell, you must count only those cells that touch the lower and left boundaries.

#### **CAUSES OF LOW MEMBERS OF WBCs (leukopenia)**

- a) Radiation treatment(cancer)
- b) Certain viral illness(HIV)

- c) Bone marrow deficiency
- d) Disease of the liver

#### **CAUSES OF high MEMBERS OF WBCs (leukocytosis)**

- a) Cigarette smoking
- b) Blood disease
- c) Tissue damage
- d) Several Mental disease.

#### **OBSERVATIONS AND CALCULATIONS**

- As observed from the four squares at haematocytes corners above where WBCs are counted from.
- $\text{WBC}/\mu\text{l} \text{ or } \text{mm}^3 = \text{No of cells in 4 squares} / 4 * \text{dilution no.} * \text{reciprocal of chamber depth}$

Dilution no= 0.5:10=20

Depth of the chamber =1/10 mm

$\text{WBC}/\mu\text{l} = \text{No.} / (4 * 20 * 10)$

Thus,

$\text{WBC}/\mu\text{l} = \text{No.} * 50$

#### **ERROR**

Has inherent of about +/- 10

#### **INTERPRETATION OF LEUKOCYTES COUNTS**

There are physiological factors to be considered such as-

1. Age
2. Stage of digestion
3. Stage of stress cycle
4. Stage of pregnancy
5. Degree of excitement.

#### **PLATELET COUNT**

For counting of total platelets cells, the same procedure and formula as that of RBCs

The only difference lies in the diluting solution

Diluting solution for platelet counting.

1% ammonium oxalate prepared by( 1g ammonium oxalate in 100 ml of diluted water)

Normal Platelet count (Thromcytosis):

Range: 140000-450000 cells per  $\mu\text{l}$

### **Causes of low platelet counts:-**

1. Cancer
2. Anaemia
3. Certain viral infection
4. Certain respiratory disorder

### **Cause of high platelets count:-**

1. Bleeding'
2. Cancer
3. Surgery
4. Certain types of anaemia
5. Certain inflammatory disease
6. Iron deficiency

### **PROCEDURES**

1. Counting chamber and the pipette should be clean and dry.
2. Finger tip and pricking lancet should be sterile.
3. Blood should freely come out without squeezing.
4. Be careful to prevent clotting of blood inside the pipette.
5. Blood should be properly mixed with the diluting fluid.
6. Cell should be settled down and more or less evenly distributed for surface counting.

### **CONCLUSION**

From this laboratory session , we learnt how to carry out a total lobe count using a haemocytometer and possible causes of leukocytosis and leukopenia conditions.

**By:- SATWIK SRIMANSU SAHOO**

**ROLL NO. -120BM0014**