Blood pressure

Definition

- **The pressure exerted by the blood against the inner walls of the blood vessels, especially the arteries. It varies with**
- The strength of the heartbeat,
- The elasticity of the arterial walls,
- The volume and viscosity of the blood,
- and a person's health, age, sex and physical condition.

Cardiac Output

- **Cardiac output** is the volume of blood pumped by the heart per minute (mL blood/min). Cardiac output is a function of **heart rate** and **stroke volume**.
- **The heart rate** is simply the number of beats per minute.
- **The stroke volume** is the volume of blood, in milliliters (mL), pumped out of the heart with each beat. Increasing either heart rate or stroke volume increases cardiac output.
- Cardiac Output in mL/min = **heart rate (beats/min) X stroke volume (mL/beat)**
- An average person has a resting heart rate of **70 beats/minute** and a resting stroke volume of **70 mL/beat**. The cardiac output for this person at rest is:
- Cardiac Output = 70 (**beats/min**) X 70 (**mL/beat**) = 4900 **mL/minute**.
- The total volume of blood in the circulatory system of an average person is about **5 liters (5000 mL)/min.**

Peripheral vascular resistance(PVR)

- refers to the resistance that produced by blood vessels and must be overcome to push blood through the circulatory system and create flow.
- Systemic vascular resistance is used in calculations of **blood pressure**, **blood flow,** and **cardiac function**.

- Total peripheral resistance (TPR) determined by **diameter of the blood vessels and viscosity of blood.**
- **Vasoconstriction** (i.e., decrease in blood vessel diameter) increases SVR,
- **Whereas vasodilation** (increase in diameter) decreases SVR.

- **Systolic pressure** – the blood pressure during the contraction of the left ventricle of the heart
- **Diastolic pressure** – the blood pressure after the contraction of the heart while the chambers of the heart refill with blood
- **Arterial pressure** – the pressure of the circulating blood on the arteries; "arterial pressure is the product of cardiac output and peripheral vascular resistance"
- **Venous pressure** – the pressure exerted on the walls of the veins by the circulating blood

objective

- Measurement of blood pressure provide us with information on the heart pumping efficiency and the condition of the systemic blood vessels.
- In general ,the **systolic blood pressure** indicates the force of contractions of the heart.
- Whereas the **diastolic blood pressure** indicates the conditions of the systemic blood vessels.

- Arterial adult blood pressure is considered normal at **120/80** where the first number is the **systolic pressure** and the second is the **diastolic pressure** (systolic/diastolic).
- **pulse pressure** : the difference between the systolic and diastolic pressure. The normal value is **40** mmHg.
- When the blood pressure 120/80, the pulse pressure= **120-80 = 40 mmHg**.

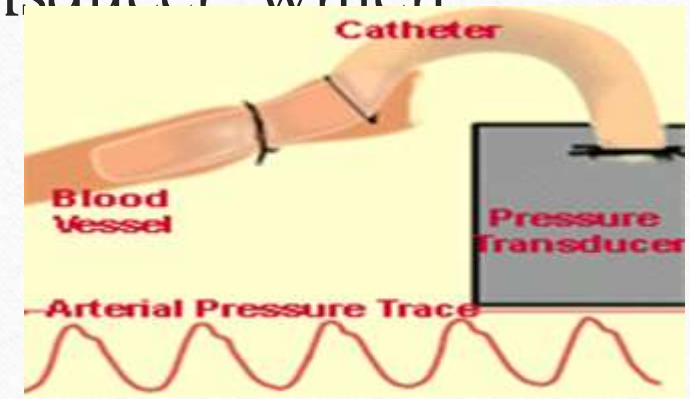
- **In practice Mean arterial pressure(map)** is the average arterial pressure during a single complete cardiac cycle.
- Map= **diastolic pressure + 1/3 (systolic pressure – diastolic pressure).**
- Map= 80 + 1/3 (120 -80)= 93 mmHg.

- The mean blood pressure is a functions of **cardiac out put** and **total peripheral resistance.**
- **Mean blood pressure= cardiac out put X total peripheral resistance .**

Measurement of blood pressure:

- **Blood pressure can be measured by several techniques :**

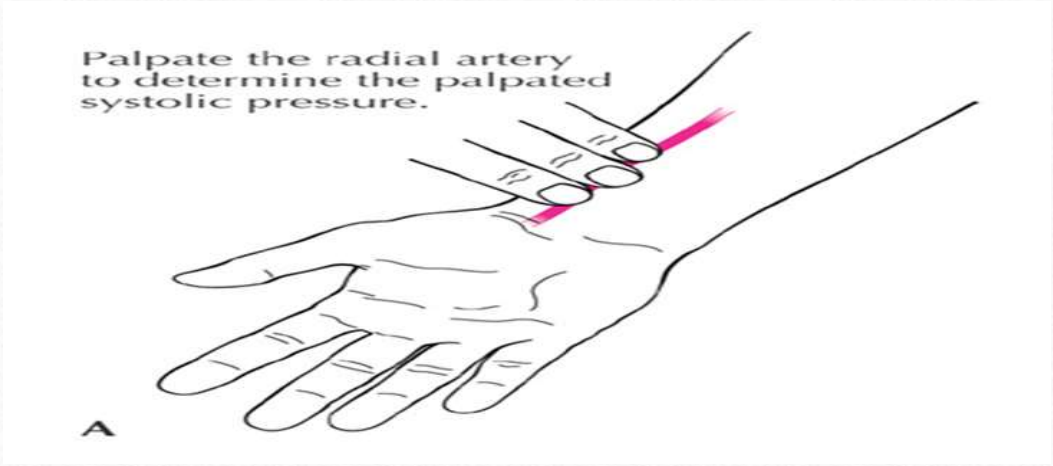
- **1-The direct method** (refer to the diagram below) involves directly inserting a tube or catheter into a blood vessel. The catheter is connected to a blood pressure transducer, which generates an electrical signal.



- **2- indirect methods involve:**
- **The first** method uses the sense of touch: it is thus called the **palpatory** method.
- **The second** method uses the sense of hearing: it is thus called the **auscultatory** method.

Determining the palpated systolic pressure and the maximum inflation level

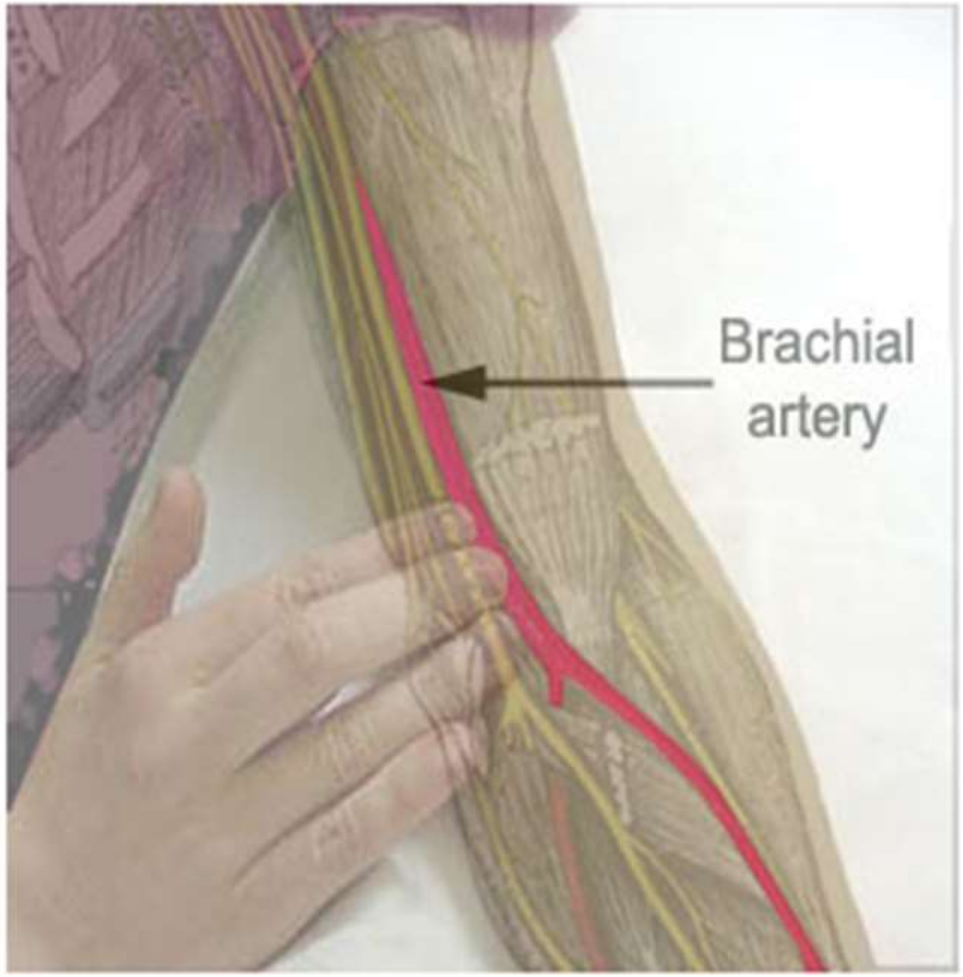
- While palpating the radial pulse, inflate the cuff until you feel the radial pulse disappear..
- Note the pressure on the manometer at this point and rapidly deflate the cuff.



- Do not leave the cuff inflated for more than 2 minutes, because it is uncomfortable and will cause a sustained increase in blood pressure.
- * The systolic Bp recorded by the palpatory method is usually around 5 mmhg lower than that obtained by the auscultatory method.
- * A major disadvantage of this method is that it can not measure the diastolic blood pressure.

- **A sphygmomanometer**, an instrument that measures pressure, is needed in both methods.
- Each sphygmomanometer consists of
  - a cuff (containing a "bladder"),
  - a rubber bulb for introducing air into the cuff
  - and the mercury for measuring the pressure in the cuff and valve.

- In addition, a stethoscope is needed for the auscultatory method.
- Note that the chestpiece of the stethoscope has both a bell and a diaphragm.


Brachial
artery

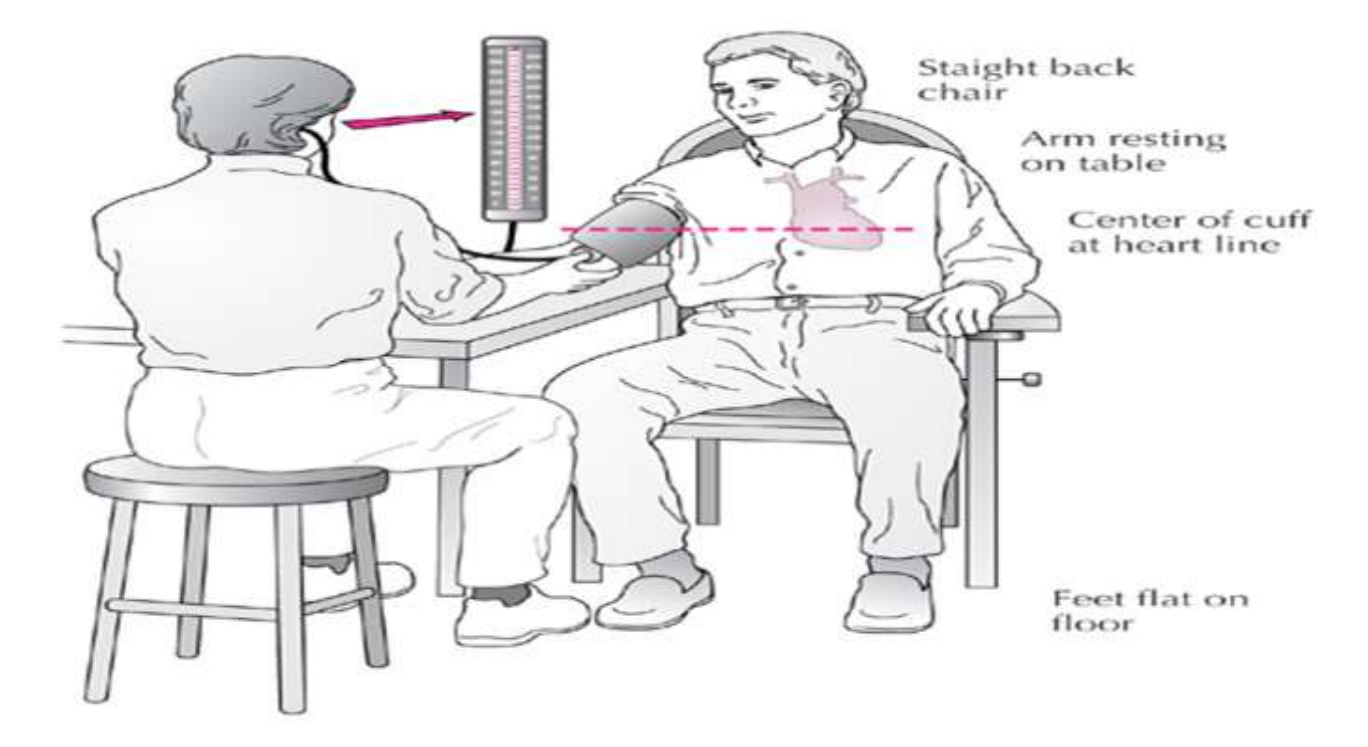
Correct positioning of the cuff

- The center of the sphygmomanometer bladder should be placed over the brachial artery. Many cuffs have some sort of marking scheme so that placement over the brachial artery – under, or just medial to, the biceps tendon – is facilitated.
- The lower border of the cuff should be ~2cm proximal to the antecubital fossa and the cuff should be firmly wrapped around the arm.

Several critical steps in measuring blood pressure

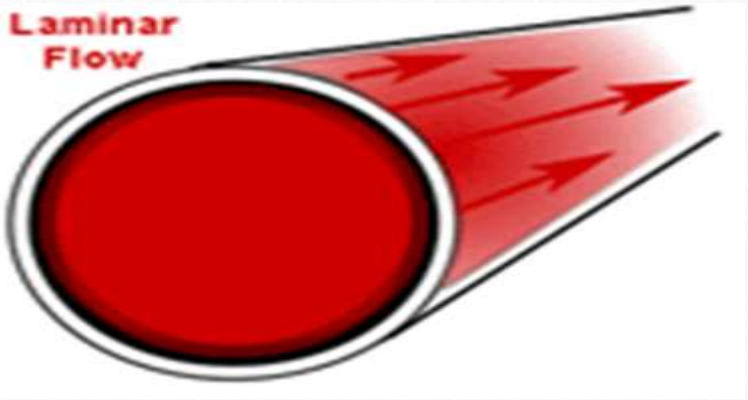
- Selection of an appropriately sized cuff.
- cuff placement.
- Proper placement of the stethoscope.
- Appropriate cuff deflation rate, and auscultation of appropriate Korotkoff sounds.

Measurement of BP in the clinic



Explanatory Notes

- The laminar flow that normally occurs in arteries produces little vibration of the arterial wall and therefore no sounds. However, when an artery is partially constricted, blood flow becomes turbulent, causing the artery to vibrate and produce sounds.



- When measuring blood pressure using the auscultation method, turbulent blood flow will occur when the cuff pressure is lesser than systolic pressure and greater than the diastolic pressure. The "tapping" sounds associated with the turbulent flow are known as **Korotkoff sounds**. Remember that these sounds are not to be confused with the heart sounds produced by the opening and closing of the heart valves.

- The sounds heard during measurement of blood pressure are not the same as the heart sounds 'lub' and 'dub' that are due to closing of cardiac valves.

- The blood pressure cuff was originally promoted as a tool to measure systolic blood pressure by the obliteration of the radial pulse. Palpation of the point at which the radial pulse was obliterated allowed for determination of the systolic blood pressure. In contrast, Korotkoff proposed listening for the appearance and disappearance of sounds to mark systolic and diastolic pressures.

Summary of the auscultatory method

- Initially the cuff is inflated to a level higher than the systolic pressure. Thus the artery is completely compressed, there is no blood flow, and no sounds are heard. The cuff pressure is slowly decreased. At the point where the systolic pressure exceeds the cuff pressure, the Korotkoff sounds are first heard and blood passes in turbulent flow through the partially constricted artery. Korotkoff sounds will continue to be heard as the cuff pressure is further lowered. However, when the cuff pressure reaches diastolic pressure, the sounds disappear.

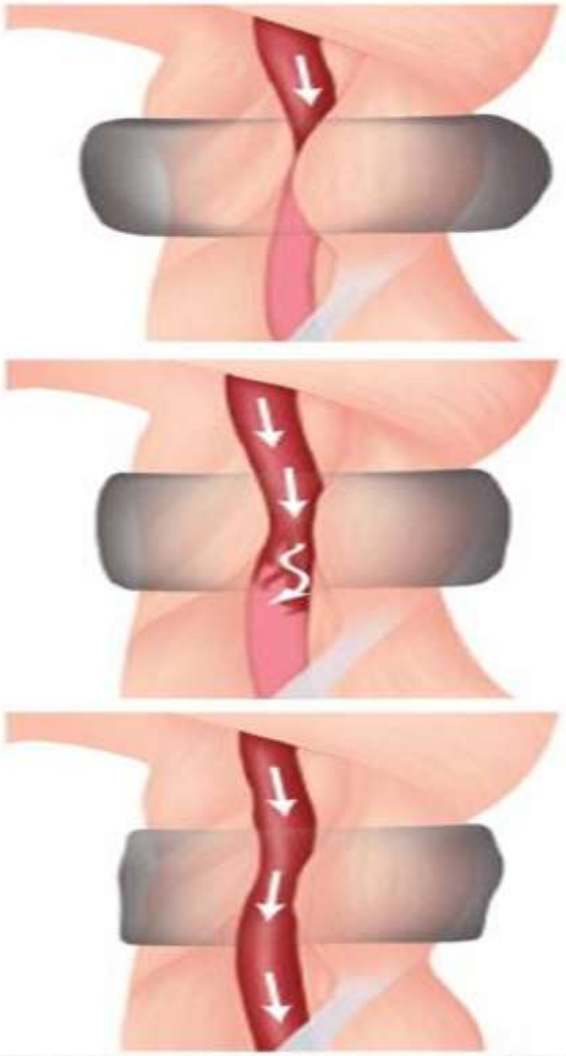
Korotkoff's sounds

- Sounds heared during the taking of a blood pressure using stethoscope and sphygmomanometer , originated by blood passage causing vibrations in the walls of the blood vessels.
- No sounds; cuff pressure above systolic pressure artery completely occluded

- **Phase 1** : apperance of fairly sharp thudding sound that increases in intensity during the next 10 mmhg of drop in presssure. the pressure when the first sound appears is the **systolic pressure**.
- **Phase 2**: the sounds become a softer murmur during the next 10-15mmhg of drop in pressure.
- **Phase 3** : the sounds become louder again and a sharpper thudding during the next 10-15 mmhg of drop in pressure.

- **Phase 4:** the sound suddenly become muffled and reduce in intesity.
- The pressure at this point is termed the diastolic pressure this muffled sound continues for another drop in pressure of 5 mmhg. after which all sound disappears. The point where the sound ceases completely is called the end diastolic pressure.

When blood pressure is 120/80:



When cuff pressure is greater than 120 mm Hg:

No blood flows through the vessel.

No sound is heard.

When cuff pressure is between 120 and 80 mm Hg:

Blood flow through the vessel is turbulent whenever blood pressure exceeds cuff pressure.

Intermittent sounds are heard as blood pressure fluctuates throughout the cardiac cycle.

When cuff pressure is less than 80 mm Hg:

Blood flows through the vessel in smooth, laminar fashion.

No sound is heard.

Errors in blood pressure readings:

- The cuff is not of the proper size: if the cuff is too small the blood pressure readings may be artefactually high. If the cuff is too big, the readings may be artefactually low.
- The cuff is positioned too loosely: the blood pressure may be artefactually high. The centre of the cuff bladder is not positioned over the brachial artery.
- The cuff is inflated slowly: a slow inflation causes venous congestion, which in turn causes the Korotkoff sounds to be faint; this results in false readings with the systolic value being too low and the diastolic reading too high.

- If the cuff is **re-inflated** immediately after an initial reading (trying to re-check the reading): a rapid re-inflation could cause venous distension, the Korotkoff sounds become more muffled. The initial Korotkoff sound may be missed so the systolic reading would be falsely low, and the diastolic reading would be falsely high because the last Korotkoff sounds could not be heard.

Classification of blood pressure in adults

Classification	Bp (mmHg)
Normal	Systolic: less than 120/Diastolic: less than 80
Prehypertension	120-139/80-89
Stage I hypertension	140-159/90-99
Stage II hypertension	equal or more than 160/equal or more than 100

Types of hypertension (H.W)

- Primary hypertension ?
- Secondary hypertension?
- Complication?

Factors affecting blood pressure (H.W)

- 1- age and sex
- 2-habits
- 3-exercise
- 4-gravity


THANK
YOU

Differential White Blood Cell Count test

Definition and Aim

- **A white blood cell (WBC) differential test,** measures the **number** or **percentage** of **each** of the five types of white blood cells present in blood:
- neutrophils
- Lymphocytes
- monocytes
- eosinophils
- Basophils

AIM

- To differentiate the type of WBCs.

- Each of these types is affected in a different way depending on the type of condition or disease that is affecting WBC counts
- Differential blood count is **not** a part of complete blood count (**CBC**) but is interpreted together with CBC to help support or exclude a suspected diagnosis. **For example**, the presence of anemia along with thrombocytopenia with a low or high white blood cell count may suggest bone marrow involvement by leukemia.. It's also necessary if the results from your **CBC** are **not** within the **normal range**.

- A differential count test can detect if there is **abnormal or immature cells or increase or decrease** in one type to other type and can help to diagnose an infection, inflammation, leukemia, a bone marrow disorder or an immune system disorder.

When is it ordered?

- It is performed when an individual undergoes a routine health examination or pre-surgical workup
- The test may be done when someone has general signs and symptoms of an **infection and/or inflammation** such as:
- Fever, chills
- Body aches, pain
- Headache

- 3. when there are signs and symptoms that may be related to a **blood disorder(as anemia or leukemia), autoimmune disorder, or an immune deficiency.**
- 4. It may also have been ordered periodically for people who take **chemotherapy or radiation** for cancer treatment.(to monitor the effectiveness of treatment).
- 5. Your CBC are not within the normal range

Normal Results

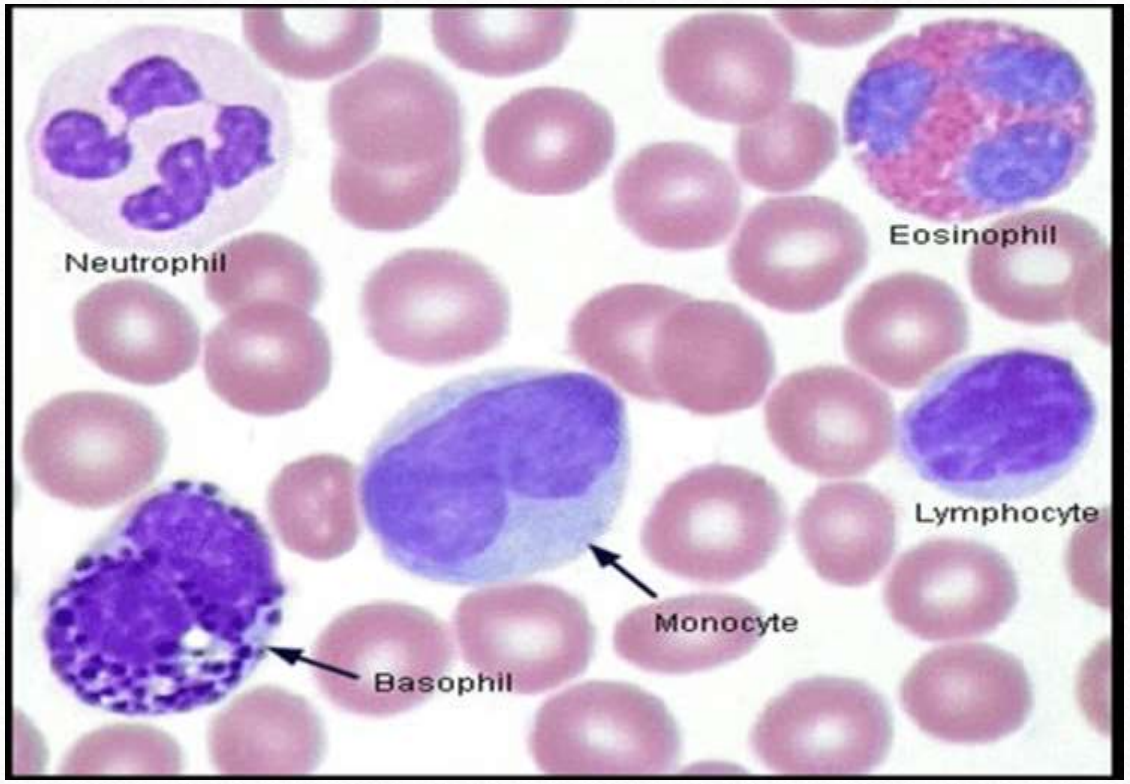
- **Reference ranges for differential white blood cell count in normal adults is as follows::**

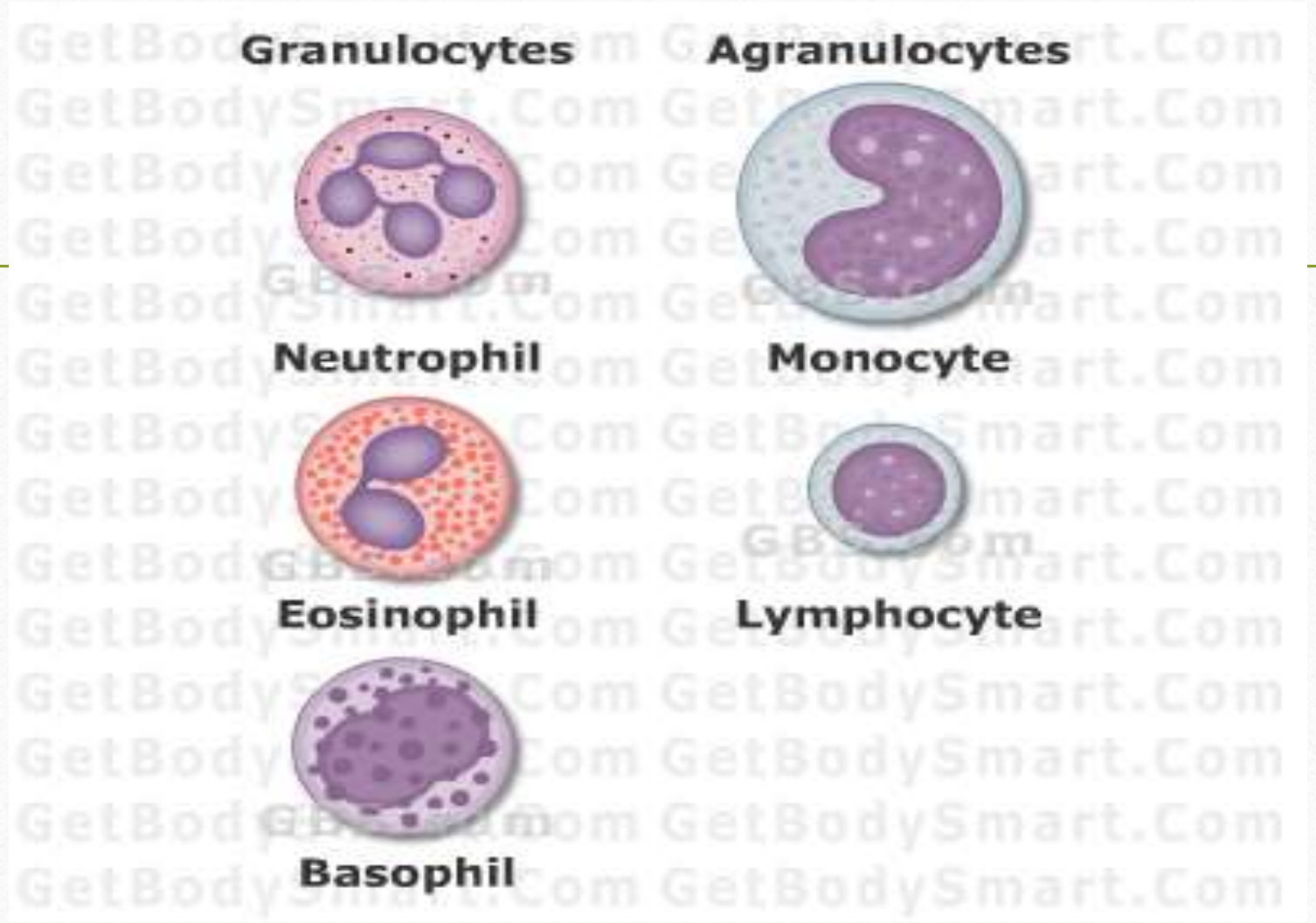
- Neutrophils: **60 to 70%**
- Lymphocytes: **20 to 40% or 20-25%**
- Monocytes: **2 to 8%**
- Eosinophils: **1 to 4%**
- Basophils: **0.5 to 1%**

Significance of high and low white blood cell counts

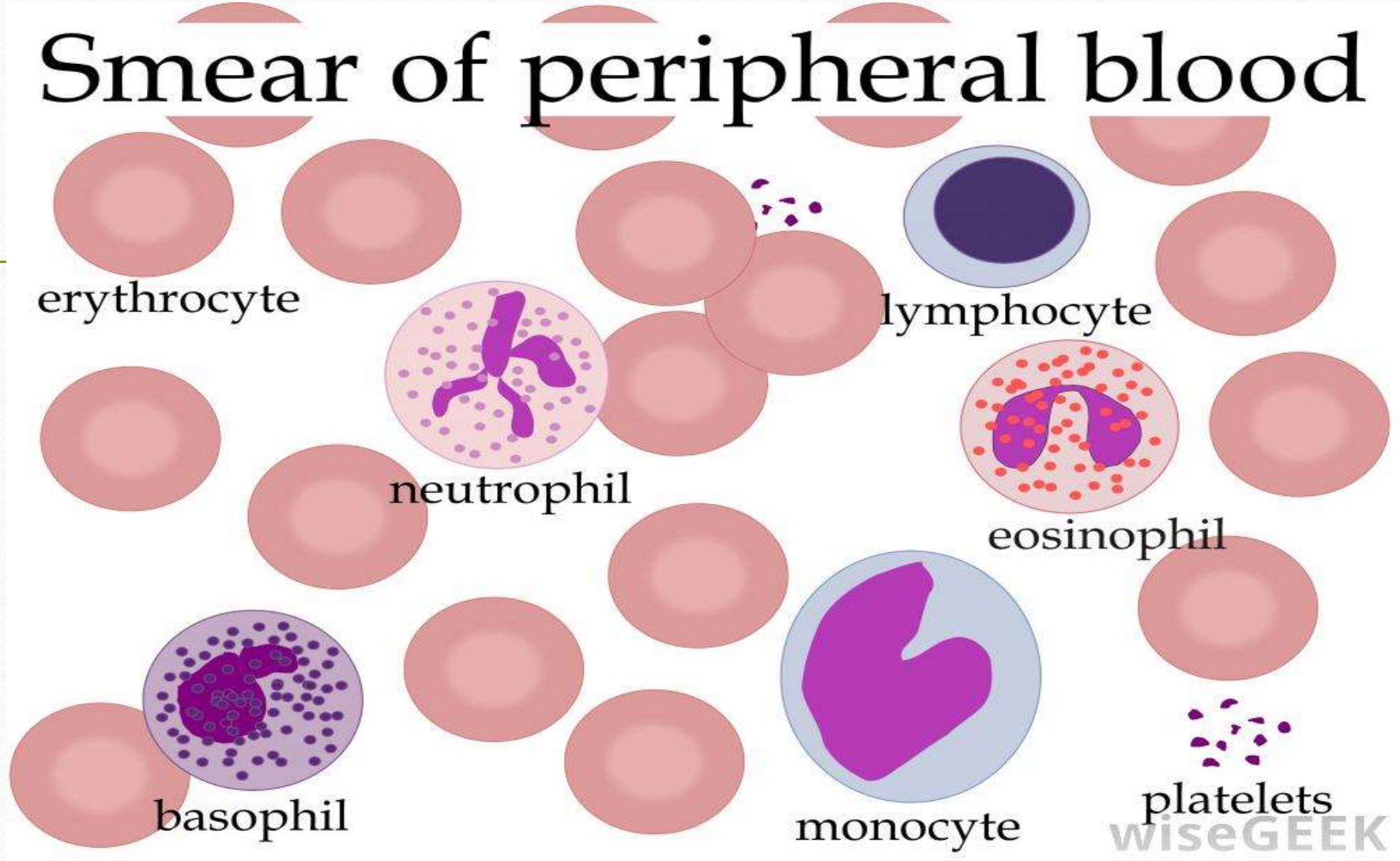
WBC type	High count may indicate	Low count may indicate
neutrophils	Bacterial infection,stress,burns	Radiation , drug toxicity , vitamin B12 deficiency
Eosinophils	Allergic reactions, parasitic infections	Drug toxicity , stress
Basophils	Allergic reactions , hypothyrodism	Pregnancy ,stress , hyperthyroidism
Lymphocytes	Viral infection	Prolonged illness, immunosuppression , treatment with cortisol
monocytes	Viral or fungal infections ,T.B ,some leukemia	Bone marrow suppression , treatment with cortisol

Normal peripheral blood smear




Granulocytes
Agranulocytes
Neutrophil
Monocyte
Eosinophil
Lymphocyte
Basophil

Smear of peripheral blood



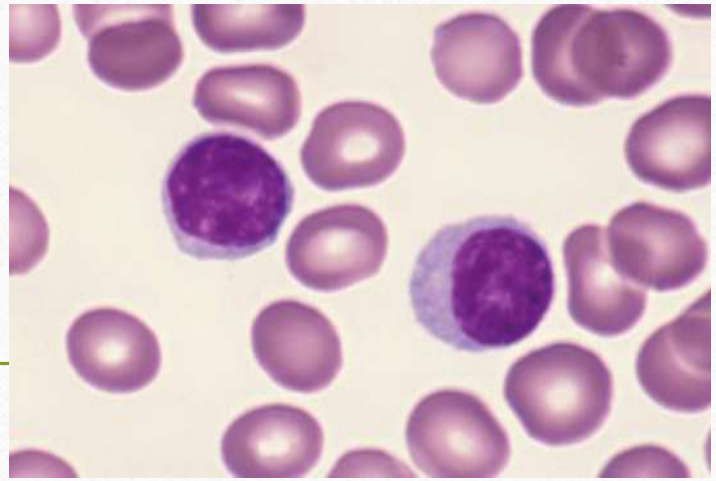
White blood cell

- **Are named according to their appearance in stained preparation**.
- Depended on presence or absence of granules in cytoplasm.
- Affinity of granules for staining with **Leishman's stain**. It consists of a mixture of **eosin** (an acidic stain), and **methylene blue** (a basic stain) in alcohol and is usually diluted and buffered before use. It stains the different components of blood in a range of shades between red and blue.

Neutrophils

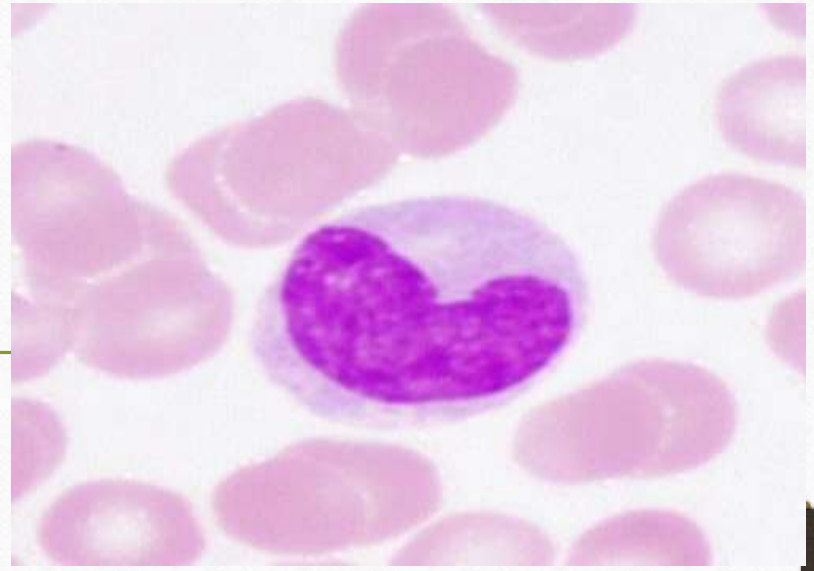
- **60-70%** of all WBCs.
- **10-12 μm** in diameter.
- * Nucleus with **2 to 5 lobes** connected by strands of chromatin, cytoplasm has very fine pale lilac granules.
- *** Function** :phagocytosis. Destruction of bacteria with **lysozyme**, and strong oxidant(free radical) such as superoxide anion($O^{\cdot --}$) ,hydrogen peroxide(H_2O_2), hydroxyl radical($OH^{\cdot -}$) and hypochlorite anion(ClO^-).

Lymphoctes



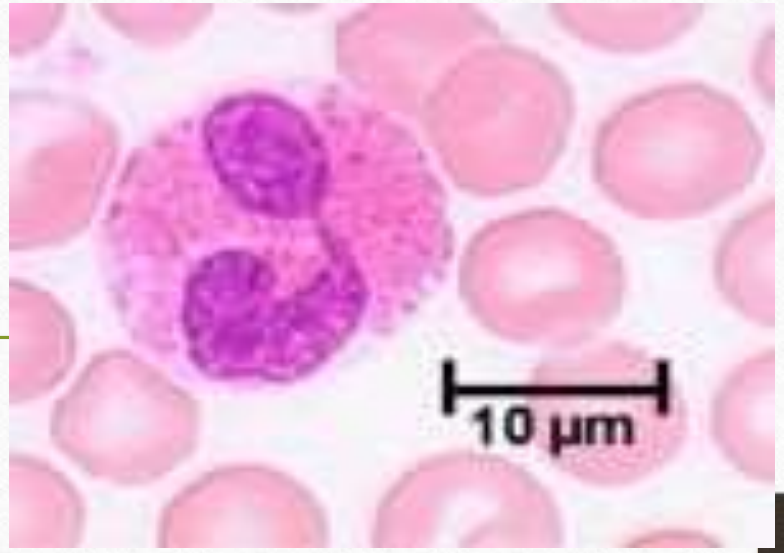
- **20 to 40%** of all WBCs.
- **Small lymphocytes** are 6-9 µm in diameter, **large lymphocytes** are 10-14 µm in diameter.
- Nucleus is round ,cytoplasm forms rim around nucleus that looks sky blue , the larger the cell the more cytoplasm is visible.
- **Function** :produces antibodies and other chemicals responsible for destroying **M.O**, regulation of immune system.

Monocytes



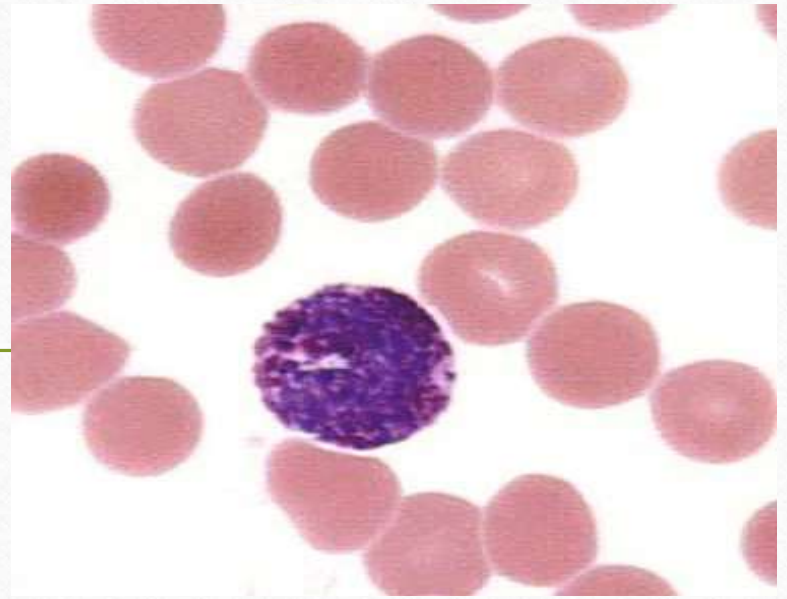
- 2-8% of all WBCs.
- 12-20 μm in diameter.
- * Nucleus round, kidney -shaped ,or horseshoe-shaped; contains more cytoplasm than does lymphocyte ; cytoplasm is blue –grey and appears foamy.
- Function : phagocytic cell in the blood ; leaves the blood and becomes a macrophage, which phagocytizes bacteria, dead cell and other debris within tissues.

Eosinophils



- **2-4%** of all WBCs.
- 10-12 µm in diameter.
- * Nucleus usually has 2 lobes connected by thick strand of chromatin, large , red-orangr granules fill cytoplasm.
- **Function** : combat effects of histamine in allergic reactions and destroy certain parasitic worms.

Basophils



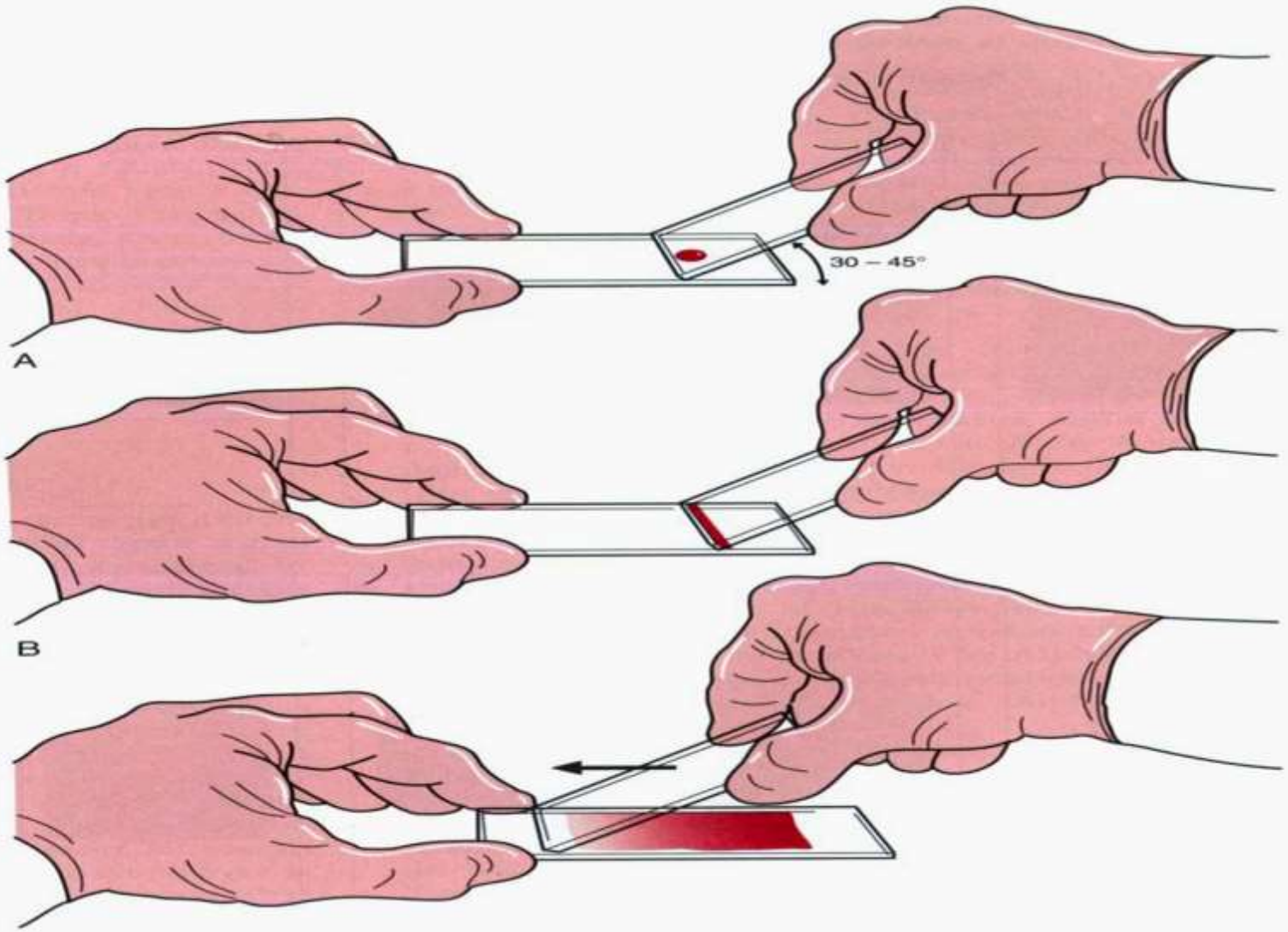
- 0.5–1% of all WBCs.
- 8–10 μm in diameter.
- * Nucleus has 2 lobes , large cytoplasmic granules appear deep blue-purple .
- Function: liberate heparin , histamine and serotonin in allergic reactions that intensify overall inflammatory response

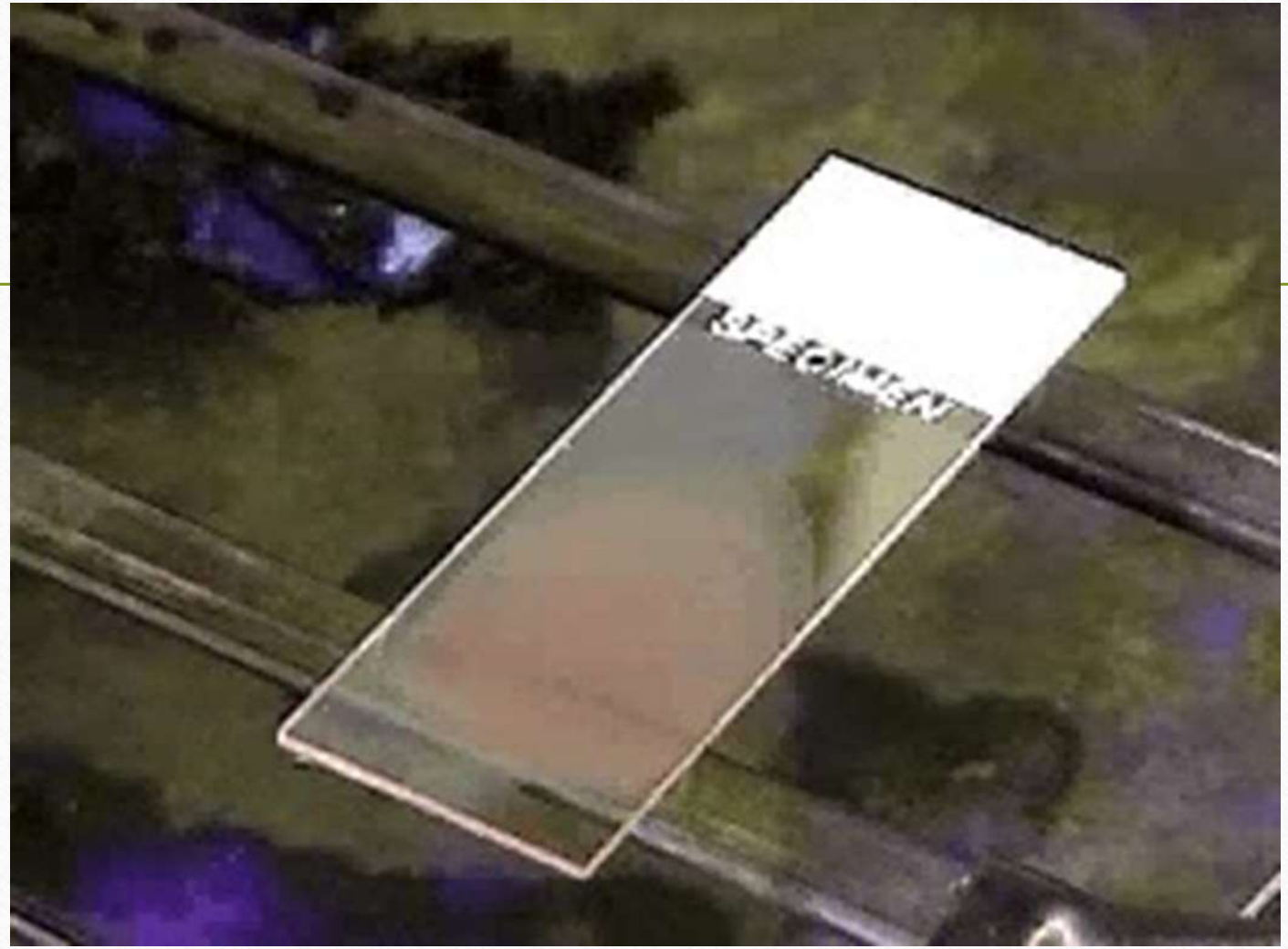
Principle:

- 1- correct preparation of the blood film.
- 2- proper staining by Leishmans stain .
- 3- accurate examination.

Procedure

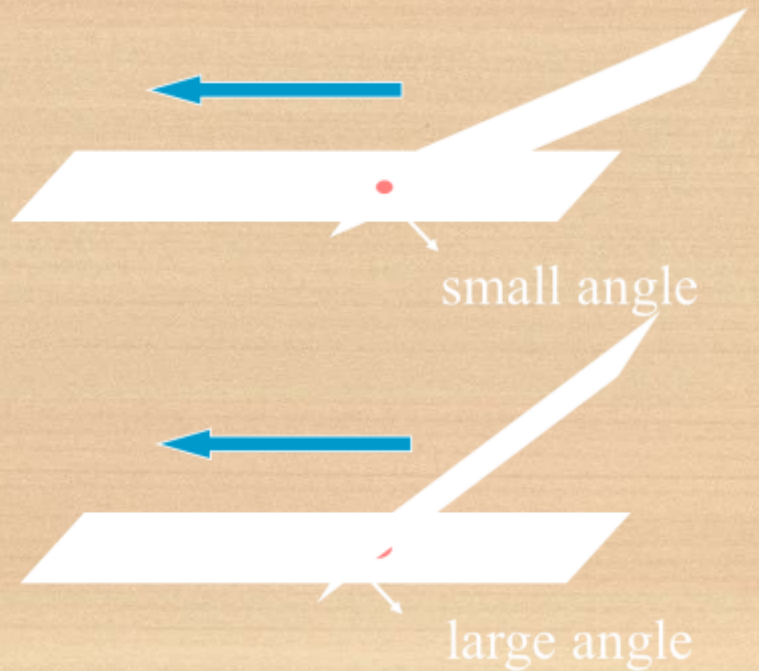
- placing a drop of blood from blood sample on a clean glass slide.
- Spreader slide using another clean glass slide at 30-40 degree angle.
- Control thickness of the smear by changing the angle of spreader slide
- Allow the blood film to air-dry completely before staining.


30 – 45°
A
B



Thickness of the film may be regulated by :

- Increasing or decreasing the angle between the slides.
- The rate of spreading.
- Adjusting the force of spreading.
- Adjusting the amount of blood.
- Thick film results when the drop of blood is large, the angle greater than 45 degrees and spreading is fast.


small angle
large angle

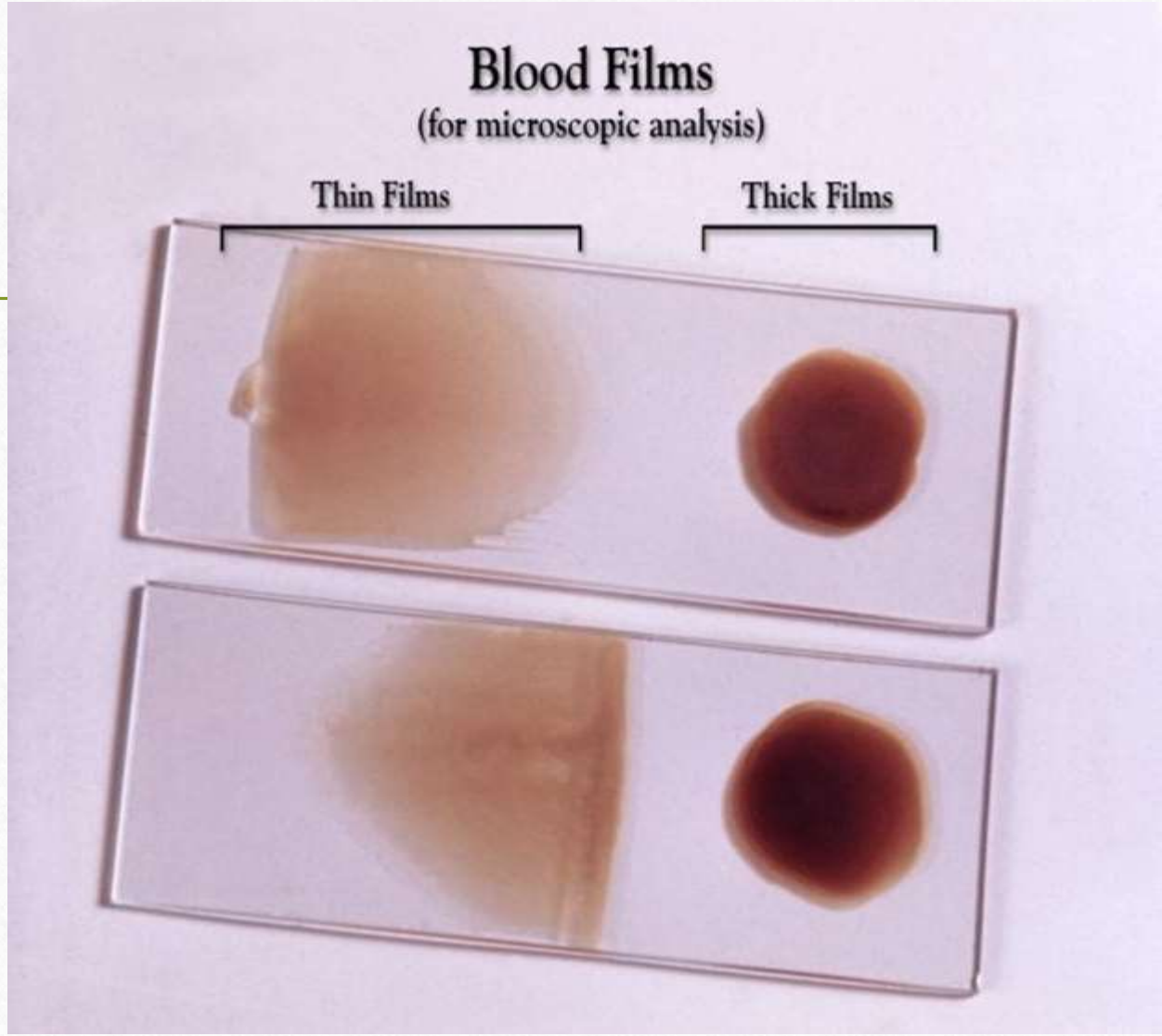
Characteristics of a good smear

- The body of the blood film should be smooth .
- The smear should be thickest at the origin and gradually thin out rather than having alternating thick and thin areas.
- A good blood film should cover half to three – fourths the length of the slids.
- Thin end of the smear should have tongue like edge

- Slides should be made in on motion and immediately, drying of the drop will lead to uneven distribution of cells in the body of the film and the larger WBCS will accumulate at the end. rouleaux of RBCS and clumping of the platelets.

Common causes of a poor blood smear

1. Drop of blood too large or too small.
2. The smear is made too rapidly.
3. Angle of spreader not constant.
4. Spreader edge is uneven or dirty.
5. Blood film with vacuoles or bubbles result from the use of dirty slides or in some cases from an excess of fat in the specimen (specimen obtained after a fattey meal).


Blood Films
(for microscopic analysis)
Thin Films
Thick Films

Examples of unacceptable blood smears?

- 1. Blood film contain thick and thin area.
- 2. Blood film contain vacuoles.
- 3. blood film not cover half to three – fourths the length of the slids.
- 4. Thin end of the smear not have tongue like edge
-

- **Leishmans stain :**

  Leishmans stain powder 0.6 gm
  Glycerin 15 ml
  Methanol q.s 300 ml

  Hot method

- Methanol, as fixing agent(fixation of cells on slide), denaturation of proteins and as a solvent to dissolve the stain.

Staining Protocol

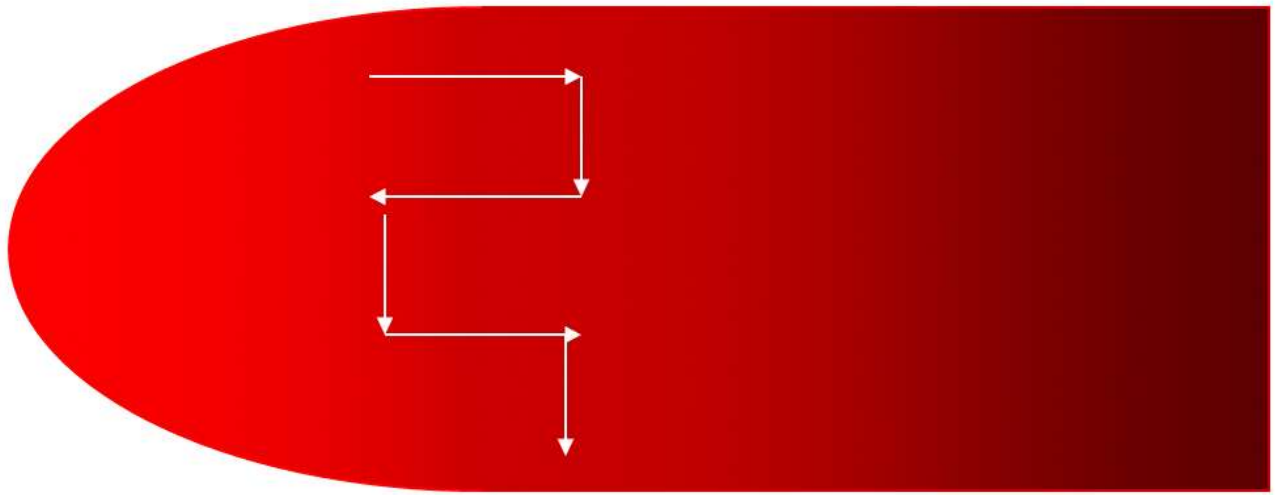
➢Blood stainings according to Leishman (covering technique)

- 1. Use smears that are **as thin as** possible and **air-dried.**
- 2. Fully cover the smears with Leishman's Stain solution. Stain for **5 minute**.
- 3. Add **same** the amount of distilled water. Incubate for at least **10-15 min**.
- 4. Rinse thoroughly with distilled water or tap water.
- 5. Dry the slides using air-dry.

Staining error

- Washed – out apperance of all the cell is caused by overwashing , understaining or underfixing , leaving water on the slide or using improper stain.
- Large amounts of precipitated stain on the film result from either improper washing or using an old stain that has started to precipitate.
- This may be corrected by ?

Observing direction:



- Observe one field and record the number of WBC according to the different type then turn to another field in the snake-liked direction
- *avoid repeat or miss some cells


Thank you!

Guidelines for Seminar Presentations

The goal of a student seminar is two-fold:

- To learn new mathematical techniques and theory via self-study.
- To learn to present this material in a proper way.

Preparing yourself for the presentation

- Before the presentation: make sure you are familiar with the room where you are giving.
- Rehearse beforehand and time yourself.
- Practice projecting your voice clearly. Vary your pitch and tone.
- A person who speaks in a monotone is boring to listen to.

- Do your last rehearsal at the latest the evening before the presentation.
- Have a good night's sleep.
- Avoid getting anxious, and don't think about it until the moment of the presentation arrives.

Giving the presentation

- Stand in a balanced position, facing the audience, feet apart.
- • Speak clearly and try not to talk too fast.

- When we are nervous, we tend to talk quickly, so try to be aware of this tendency.

- • Maintain eye contact with your audience. It will help them concentrate because they will feel more involved.
- A good technique is to divide the audience into three sections (left, middle and right) and sweep
- • Don't read straight from your paper. This is boring for your audience.

Presentations should have the following structure:

- A good introduction will capture the audience's attention.
- First greet the audience.
- Introduce yourself (even if they already know your name).
- Try starting with a question or simply saying: "todayI would like to talk to you about—"

- Tell your audience what you are going to talk about. State:
- • what your topic is, and what your presentation will cover.
- • An outline of the main points
- • Any necessary history or definition of terms.

Body

- The body of your presentation is where you develop the main points of your talk, and present examples and evidence.

Conclusion

- The conclusion is usually a summary of the main points made in the body of the talk.
- Don’t introduce any new information in the conclusion.
- Take the opportunity to show that you have covered all the points you made in your introduction.

Designing PowerPoint slides

**Slide Layout

- Do not overload slides;
- Use little text, keep text in one line;
- Use diagrams and flowcharts;
- Keep as simple as possible

Presenting Slides

- Dedicate at least two minutes per slide;
- Cut the number of slides if time is not enough;

- Keep introduction and motivation short;
- Do not spend too much time on the talk's outline (max. one minute)
- Allow more time for complex topics:
- Repeat if really important

- Use examples (a running example/more examples

Using text

- Avoid using too much text. (slides should have no more than six
- bullet points and each bullet point should be no more than six words long).
- Create bullet points which are clear summaries of key points..
- Don’t mix up your fonts and font sizes.
- Ensure that your text is at least 24pt otherwise it may be difficult to read on screen.
- Choose left align for all text to make it easier to read.

- Use bold for a clear and simple form of emphasis and headings rather than italics or underlining.

Fonts

- Font Style Should be Readable
  - Recommended fonts: Arial, Tahoma, Veranda

Font Size

✶ **The larger, the better. Remember, your slides must be readable, even at the back of the room.**

- This is a good title size Verdana 40 point
- A good subtitle or bullet point size Verdana 32 point
- Content text should be no smaller than Verdana 24 point
- This font size is not recommended for content. Verdana 12 point.

Font Size

- Combining small font sizes with bold or italics is not recommended:

  - *What does this say? Garamond Font, Italic, Bold 12pt.*
  - **This is very difficult to read. Times Font, Bold, 12pt.**
  - ***This point could be lost. Century Gothic Font, Bold, Italic, 14pt.***
  - No one will be able to read this. Gill Sans Font, Condensed Bold, 12pt

CAPITALS and Italics

- **DO NOT USE ALL CAPITAL LETTERS**
  - Makes text hard to read

- Italics
  - Used for "*quotes*"
  - Used to *highlight* thoughts or ideas
  - Used for book, journal, or magazine *titles*

Use a Template

- Use a set font and color scheme.
- *Different* **styles** are DISCONCERTING to the audience.
- You want the audience to focus on what you present, not the way you present.

Use the Same **Background** on Each Slide

- Don’t use multiple **backgrounds** in your presentation
- Changing the style is distracting

Using colour

- Be consistent.
- Ensure that all of your slides have the same or similar background images and colour schemes.
- PowerPoint’s design templates can be used for this.
- Prepare slides that use a bold colour contrast, e.g. black or deep blue text on a cream background (black
- and white can be too glaring for the audience).

- Avoid using red or green for text or highlighting as it can be difficult to read.

Avoid These Combinations

- Examples:
  - –Green on Blue
  - –Dark Yellow on Green
  - –Purple on Blue
  - –Orange on Green
  - –Red on Green

Colors

- Avoid **white** backgrounds
- The white screen can be **blinding** in a dark room.
- **Dark slides** with **light colored text** work best.

Background Colors

Remember: Readability! Readability! Readability!

This is a good mix of colors. Readable!

This is a bad mix of colors. Low contrast. Unreadable!

This is a good mix of colors. Readable!

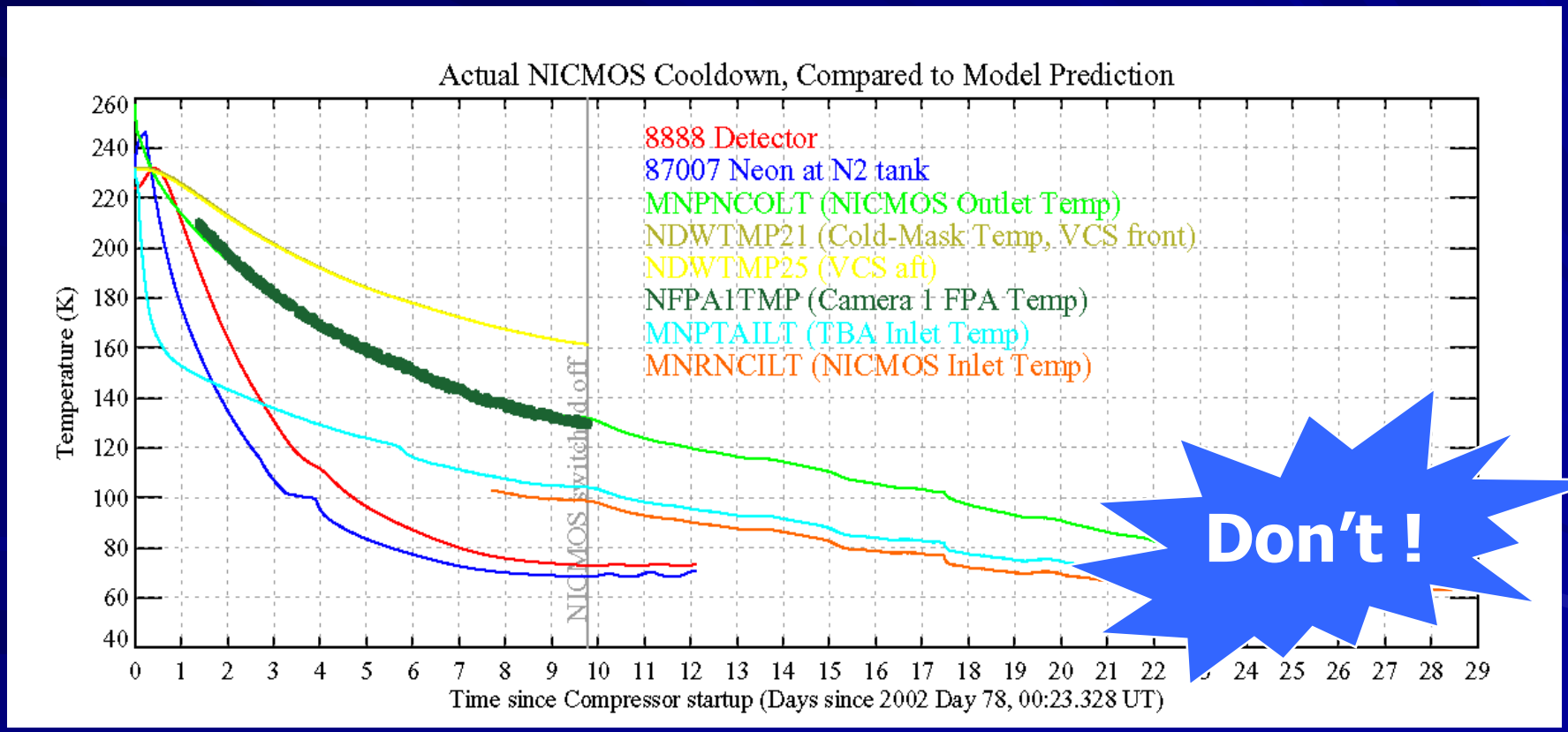
This is a bad mix of colors. **Avoid bright colors on white.** Unreadable!

Graphs and Charts

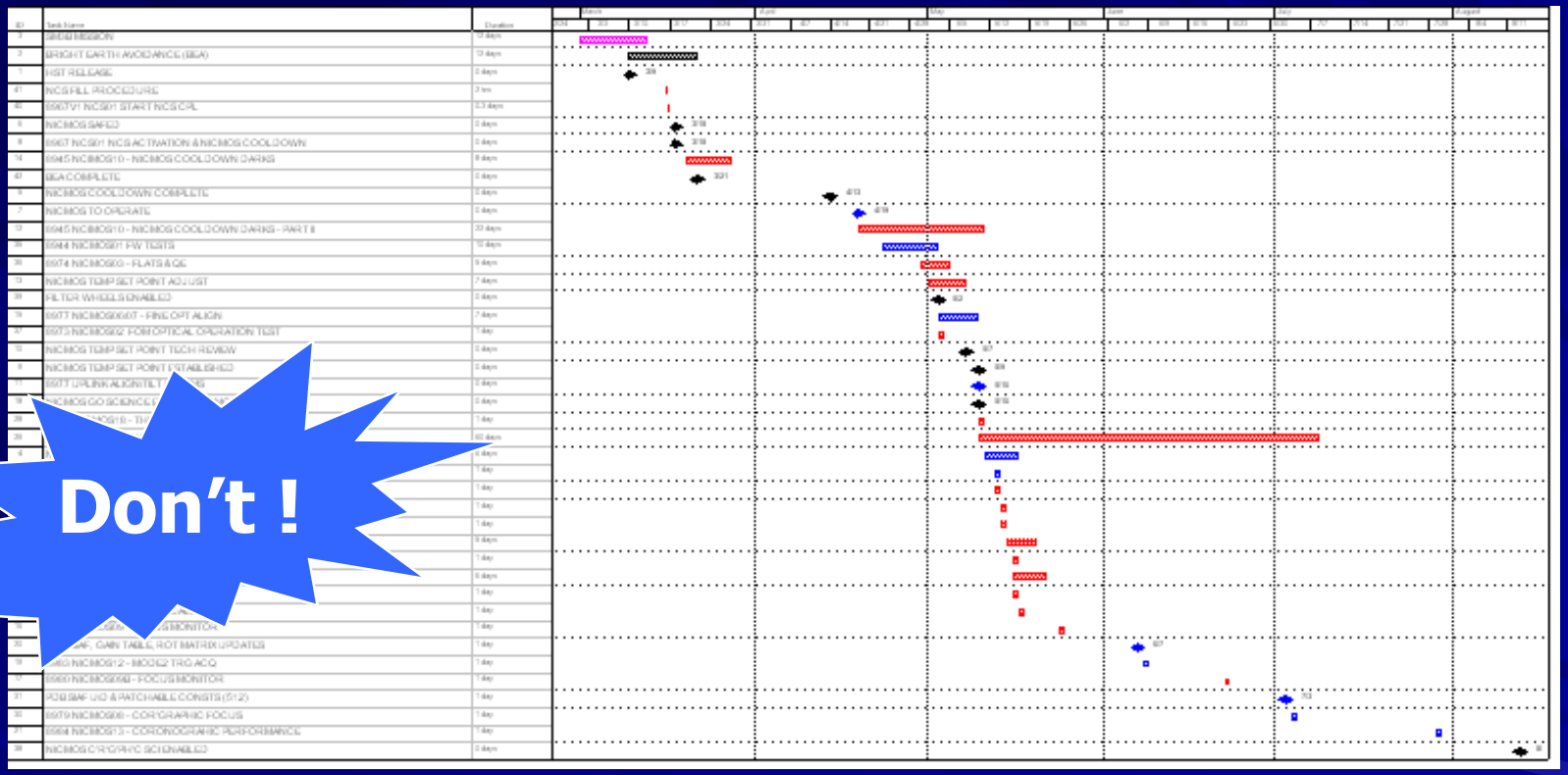
Make sure the audience can read them!

Graphics and Charts

Avoid using graphics that are difficult to read. In this example, the bright colors on a white background and the small font make the graph hard to read. It would be very difficult to see, especially in the back of a room.



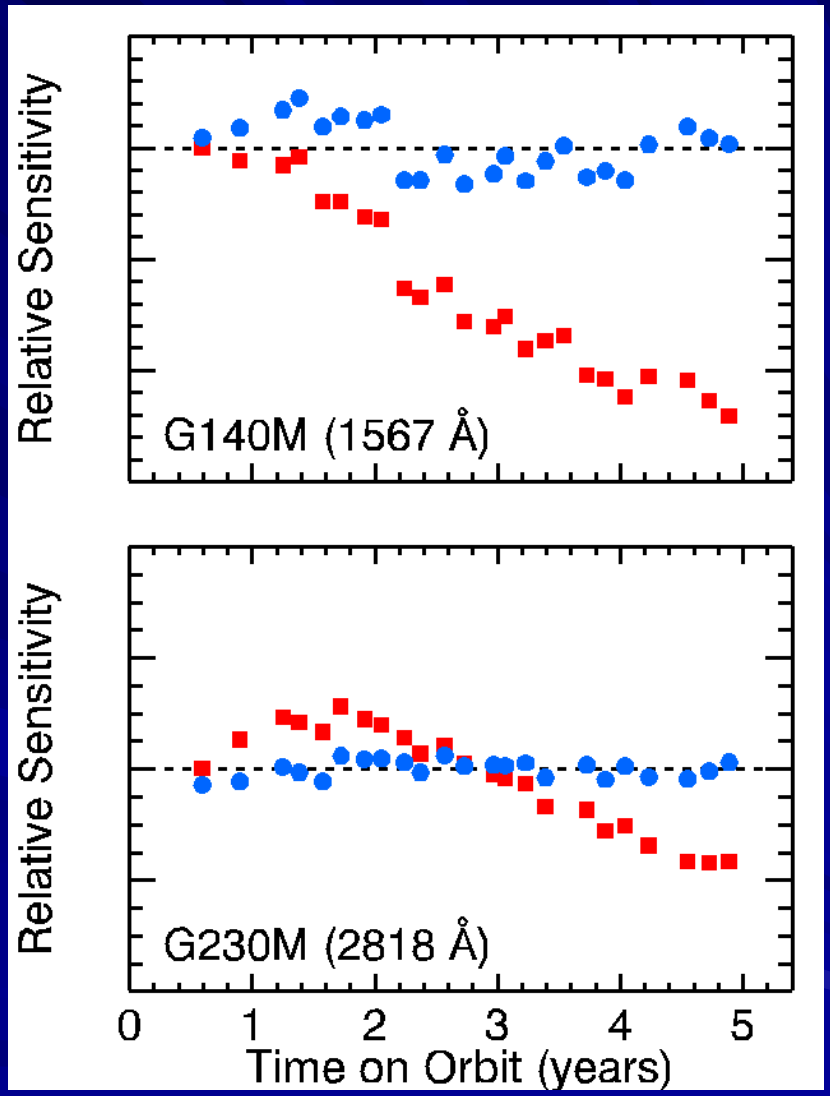
This graph contains too much information in an unreadable format.



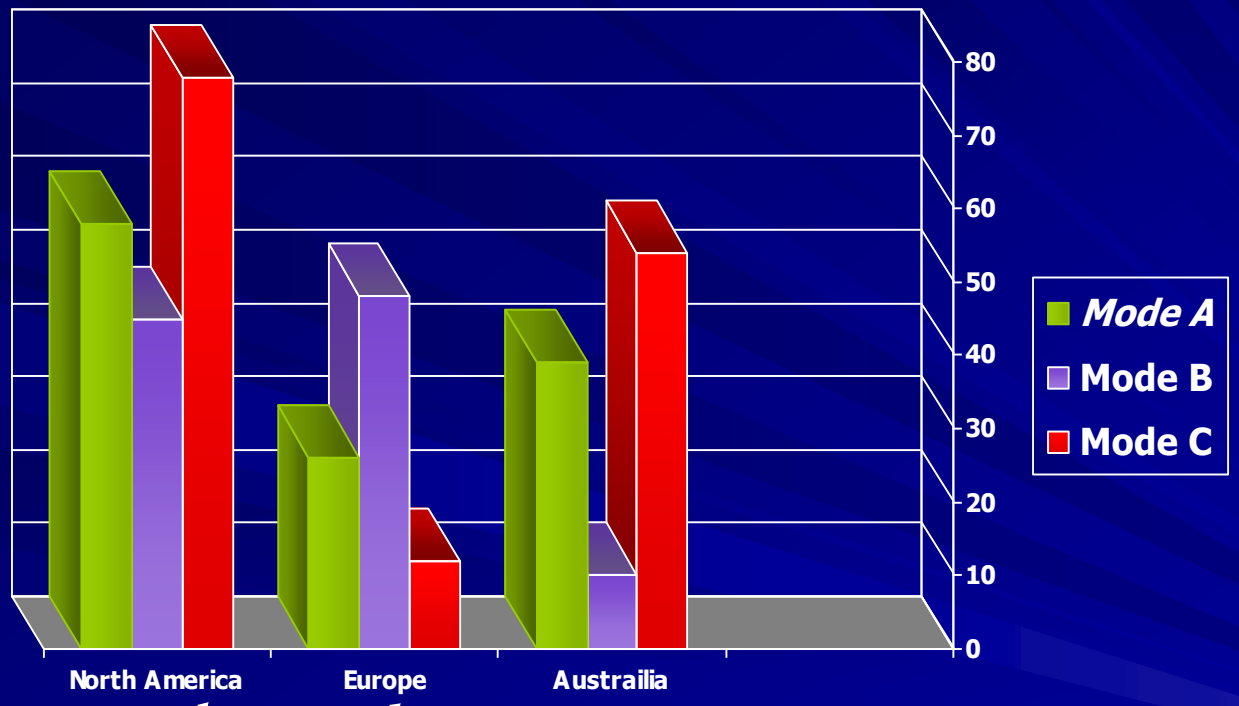
Good Graph

These are examples of good graphs, with nice line widths and good colors.

Do !

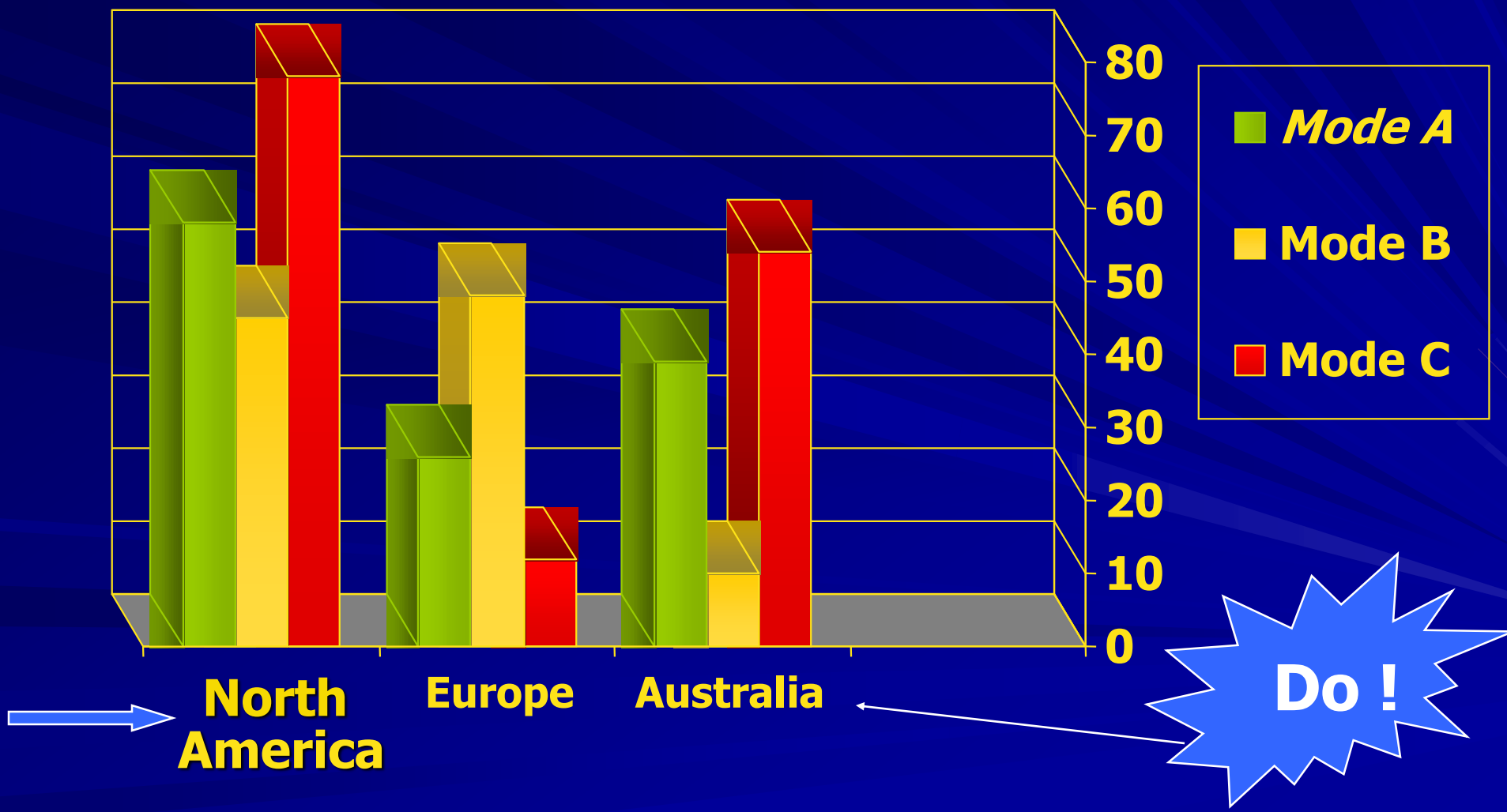


Charts and Graphs



Don't

Charts and Graphs

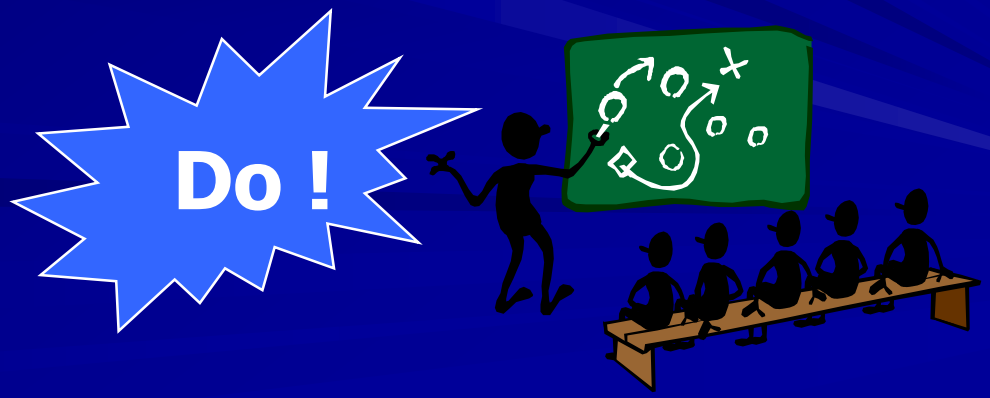


This is a good, readable table. Tables, especially large ones, should be placed on a separate slide.

4/19 Fri	109	NICMOS restarted, Ne-loop control continues
4/22 Mon	112	Change to mount[illegible] ntrol
4/23 Tue	134	Return to Ne control, Filter wheel test begins
4/24 Wed	155	Increase control temperature to allow for +2 K variations
4/25 Thur	165	Begin darks every 3rd orbit
4/26 Fri	174	DQE test visit 1; Control temp +0.5 K

Illustrations

- Use only when needed, otherwise they become distracters instead of communicators
- They should relate to the message and help make a point
- Ask yourself if it makes the message clearer
- Simple diagrams are great communicators



seminar topics

1-**Parathyroid hormone and physical exercise.**

2- Water as an essential nutrient: the physiological basis of hydration.

3- Leptin, its implication in physical exercise and training.

4- **-Melatonin; from pineal gland to healthy foods.**

5- Thyroid gland.

6- Adrenal gland.

Thank you

Total erythrocytes (RBC)count

- **This test is almost always a part of a complete blood count (CBC) test. A CBC test measures the number of all types of components in the blood, including:**

- red blood cells
- white blood cells
- hemoglobin
- hematocrit
- platelets

Definition and Aim

- **A red blood cell count:** is a blood test that used to find out how many red blood cells (RBCs) in blood. It's also known as an erythrocyte count.
- **Aim:** To measure or find out the number of red blood cells in one cubic millimeter of blood

- This test is **important** because RBCs contain hemoglobin, which carries oxygen to body's tissues. The number of RBCs in blood can affect how much oxygen the tissues received, because tissues need oxygen to function effectively.

When is it ordered?

- As part of a routine physical examination or as part of a pre-surgical workup.
- When someone has signs and symptoms that associated with low RBS count, which can be seen in (anemia), include:

- Weakness or fatigue
- Lack of energy
- Paleness
- Some signs and symptoms that may associated with a high RBC count (erythrocytosis) can be seen in (Polycythemia) include:

- Disturbed vision
- Headache, dizziness
- Flushing
- Enlarged spleen

- **This test may also be performed on a regular basis to monitor people who have been diagnosed with conditions such as**

1. blood disorders (chronic anemia, and polycythemia.) , kidney disease, bleeding problems,
2. Chemotherapy or radiation therapy often decreases bone marrow production of all the blood cells. Thus, a CBC is typically ordered at regular intervals when monitoring people who are undergoing treatment for cancer.

➢ Normal range for RBCs Count are as follows:

- Women **4.0–5.5** million/mm3
- Men **4.5–6.0** million/mm3
- Newborn **5.0–6.5** million/mm3
- Pregnancy: slightly lower than normal adult values
- Children: **3.8 – 5.5 million**

Abnormal results
if RBC count is higher than normal, is due to:

- cigarette smoking.
- congenital heart disease.
- Dehydration.
- renal cell carcinoma, which is a type of kidney cancer.
- pulmonary fibrosis.
- polycythemia vera, which is a bone marrow disease that causes overproduction of RBCs and is associated with a genetic mutation.
- higher altitude, your RBC count may increase for several weeks because there's less oxygen present in the air.
- Certain drugs, such as gentamicin and methyldopa, can also increase RBC count.

Lower-than-normal numbers of RBCs may be due to:

- Anemia
- Bleeding

- Bone marrow failure (for example, from radiation, toxins, or tumor)
- Deficiency of a hormone called erythropoietin (caused by kidney disease)
- RBC destruction (hemolysis) due to transfusion, blood vessel injury.
- Leukemia
- Malnutrition or nutrition deficiencies of (iron, copper, folic acid, vitamin B6, or vitamin B12).
- multiple myeloma (Bone marrow cancer)

- Overhydration
- Pregnancy
- Drugs that can decrease the RBC count as example:

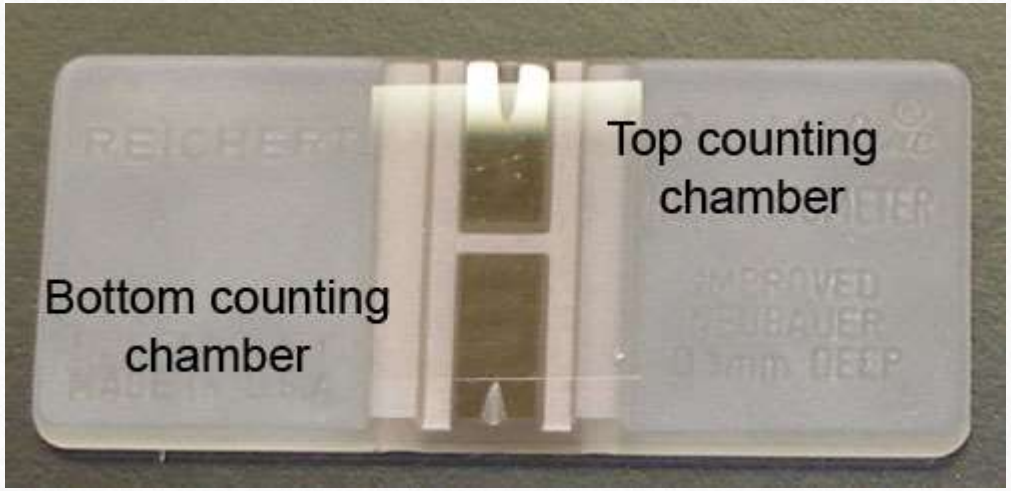
- Chemotherapy drugs
- Chloramphenicol
- Quinidine

Principle

- It consists of an accurate dilution of a measured quantity of blood with a fluid which is **Isotonic** with the blood and prevents coagulation.
- Haemocytometer apparatus includes:

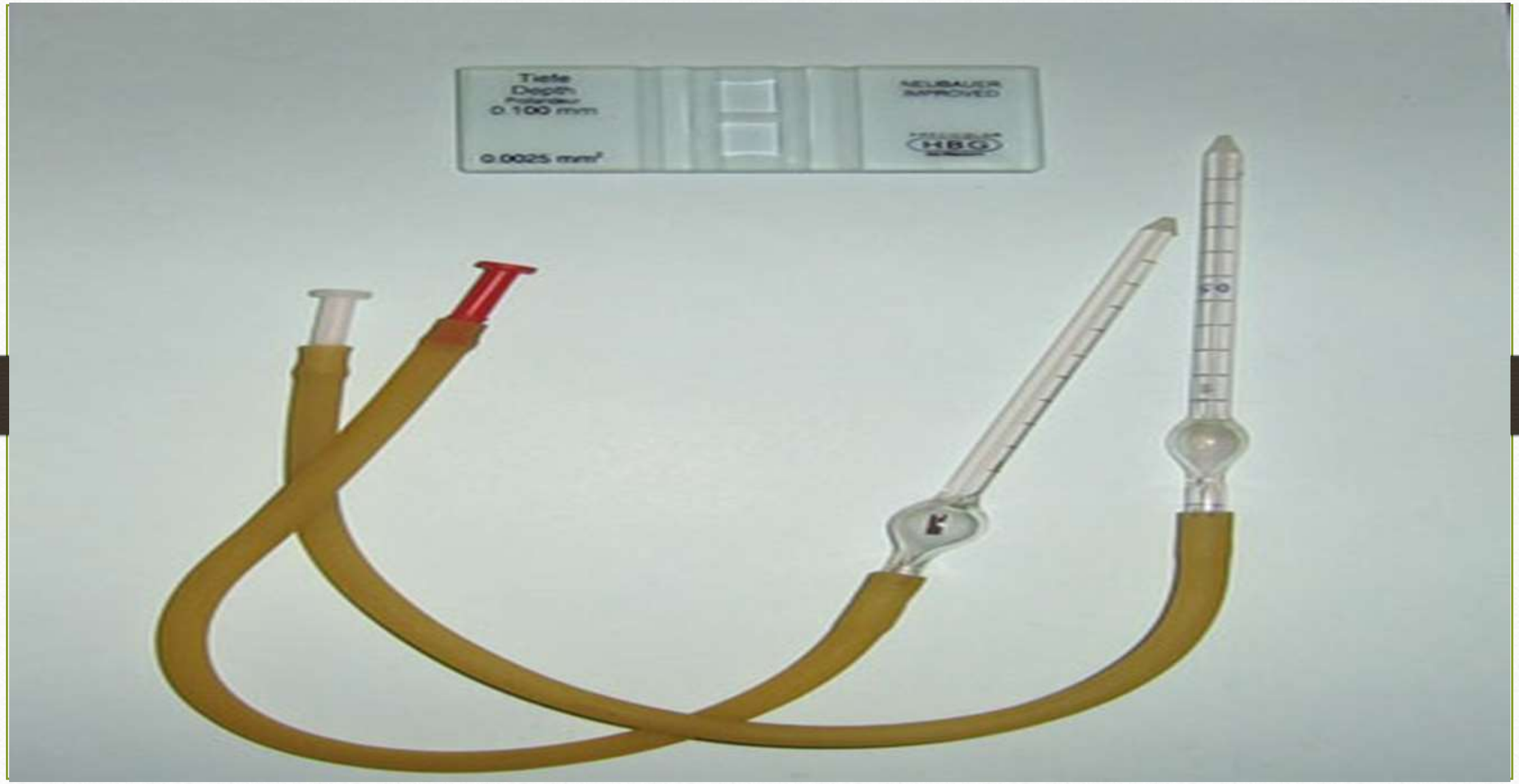
1. Neubauer's slide
2. Cover slip
3. RBC pipette
4. WBC pipette


HAEMOCYTOMETER

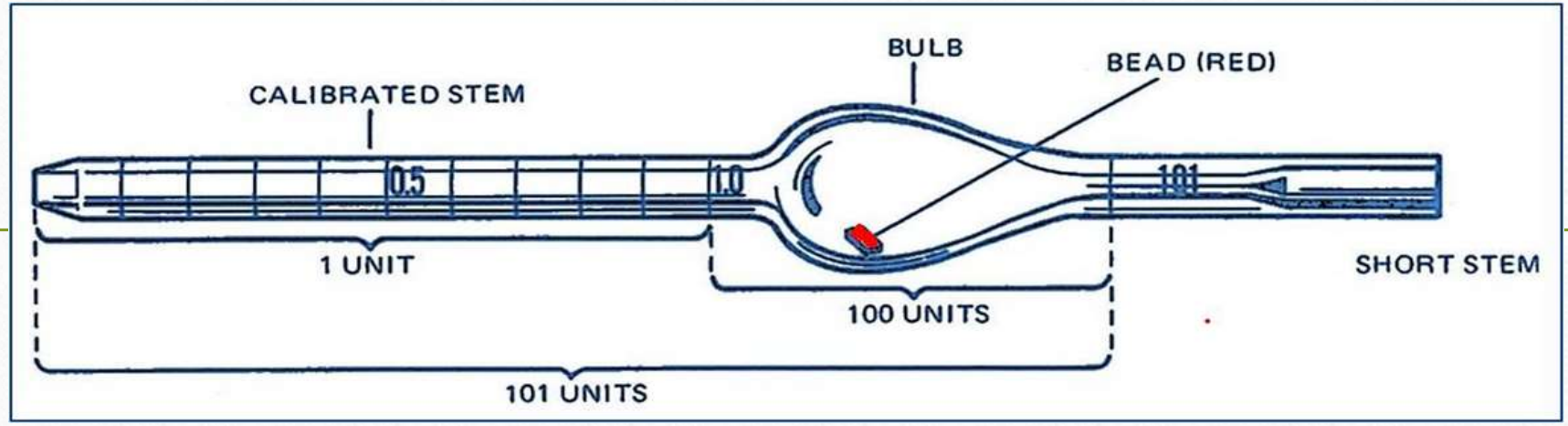

Top counting chamber
Bottom counting chamber

Differences between RBC and WBC pipettes

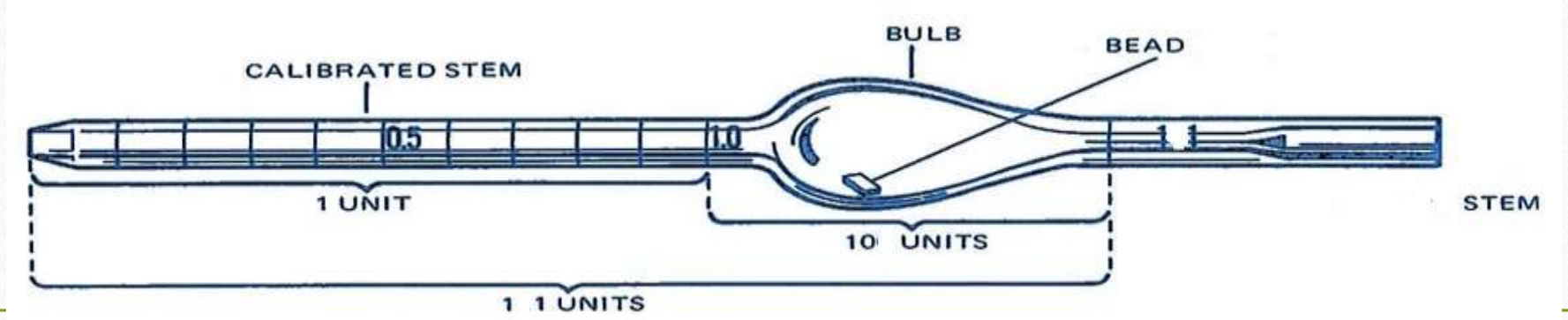
RBC pipette	WBC pipette
1-It has a red bead. 2-It has graduations up to mark 101. 3-Size of bulb is larger	1-It has a white bead. 2-It has graduations up to mark 11. 3-Size of bulb is smaller.


Tiefe
Depth
0.100 mm
0.0025 mm²
NEUBAUER
IMPROVED
HBG

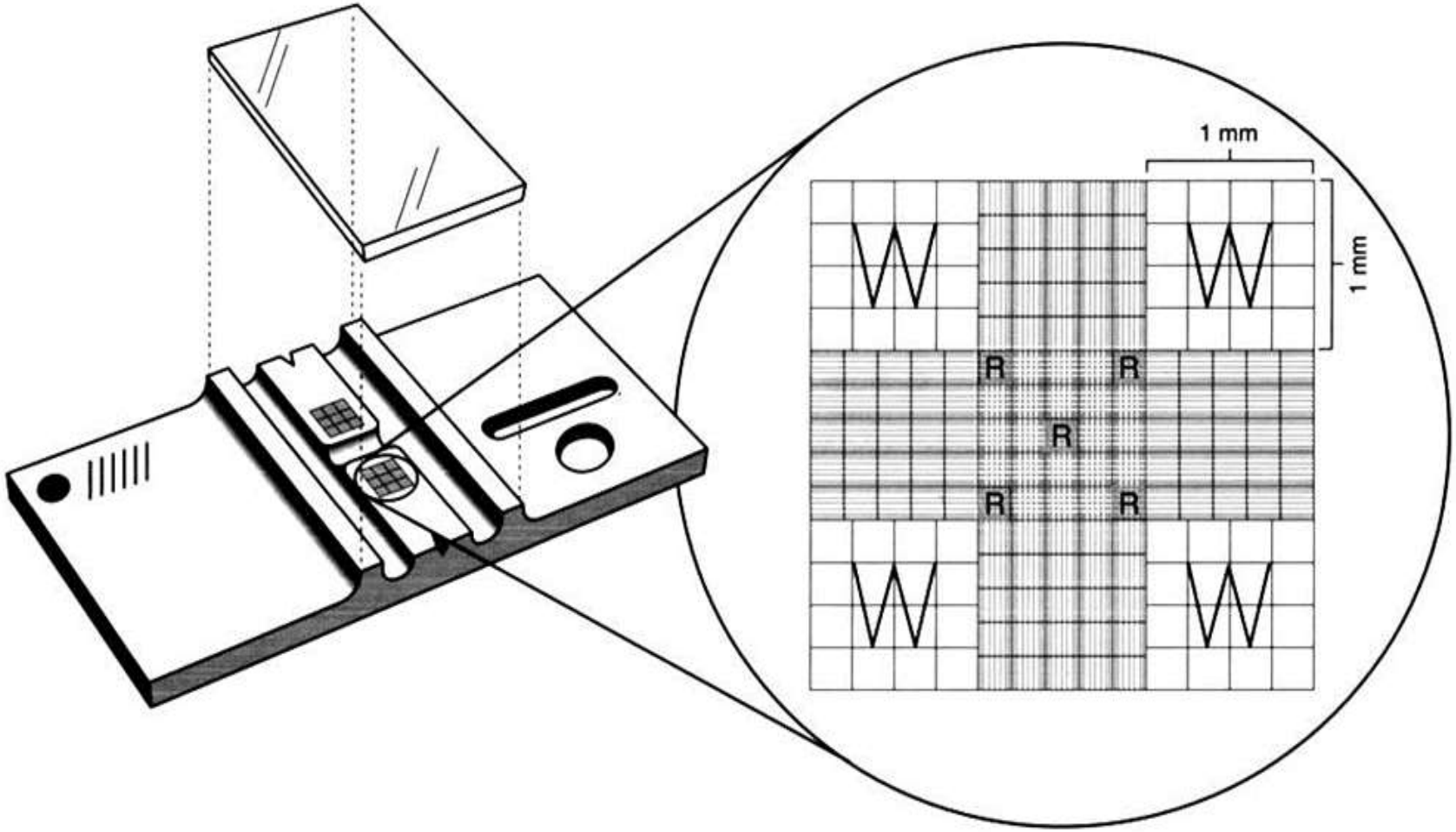
RBC PIPETTE

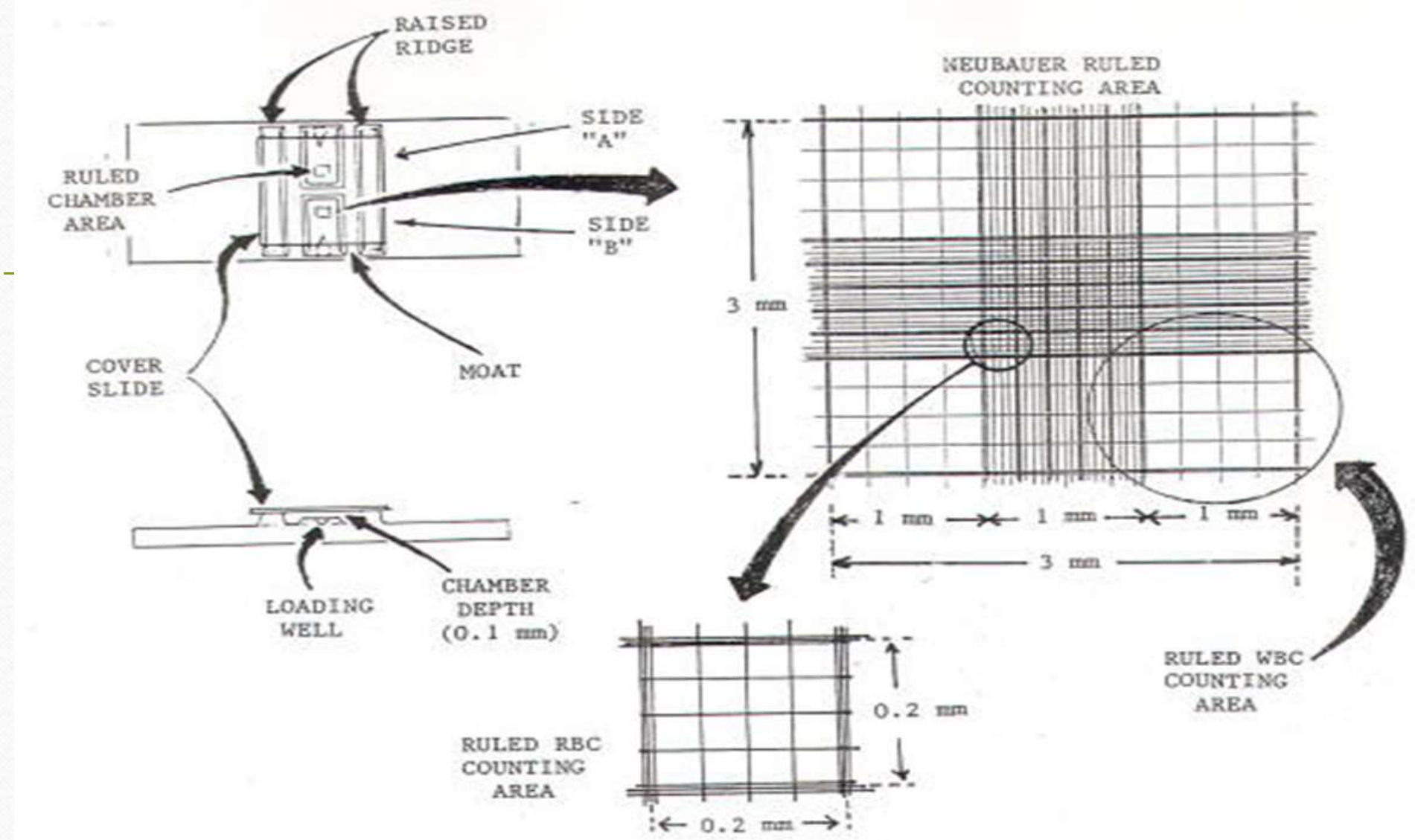


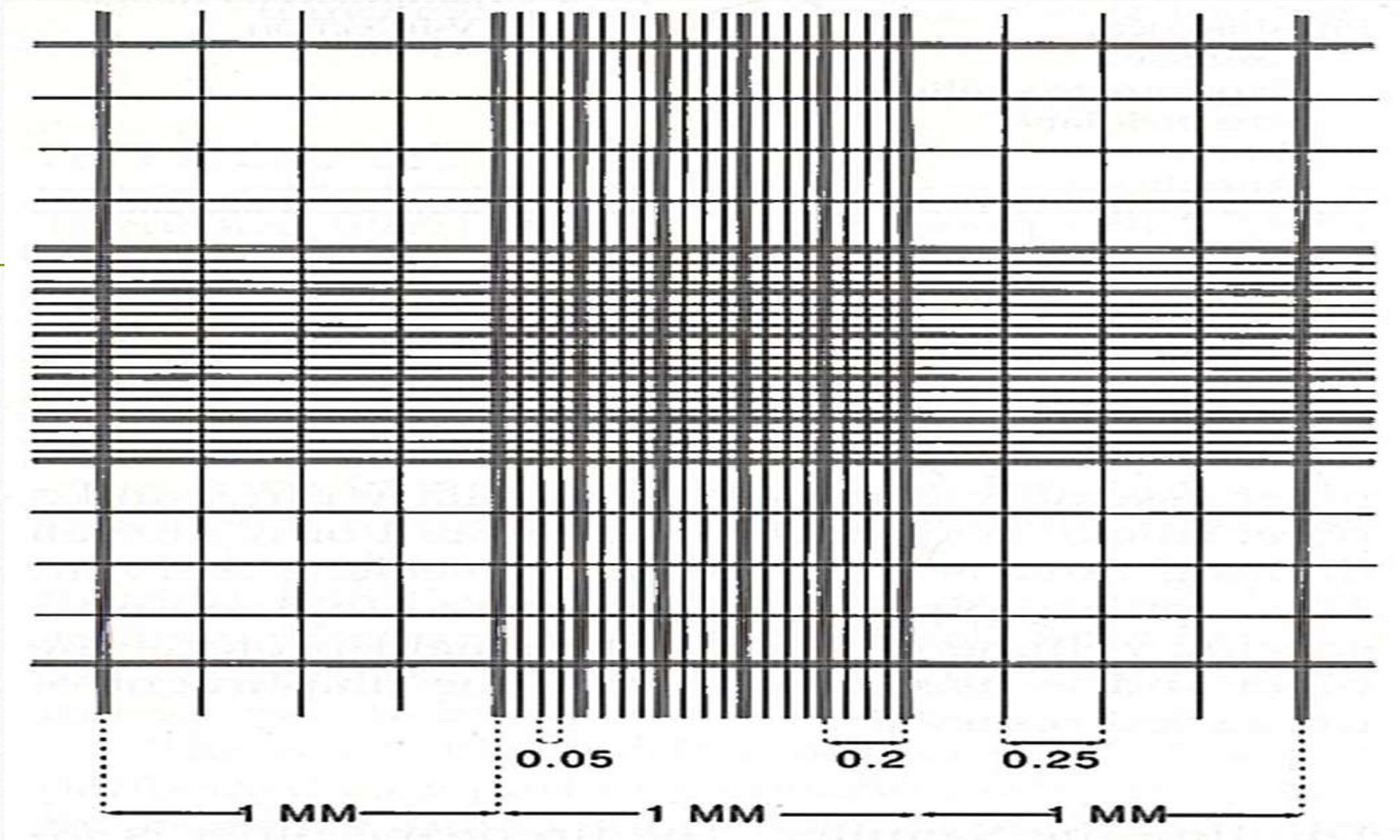
WBC PIPETTE

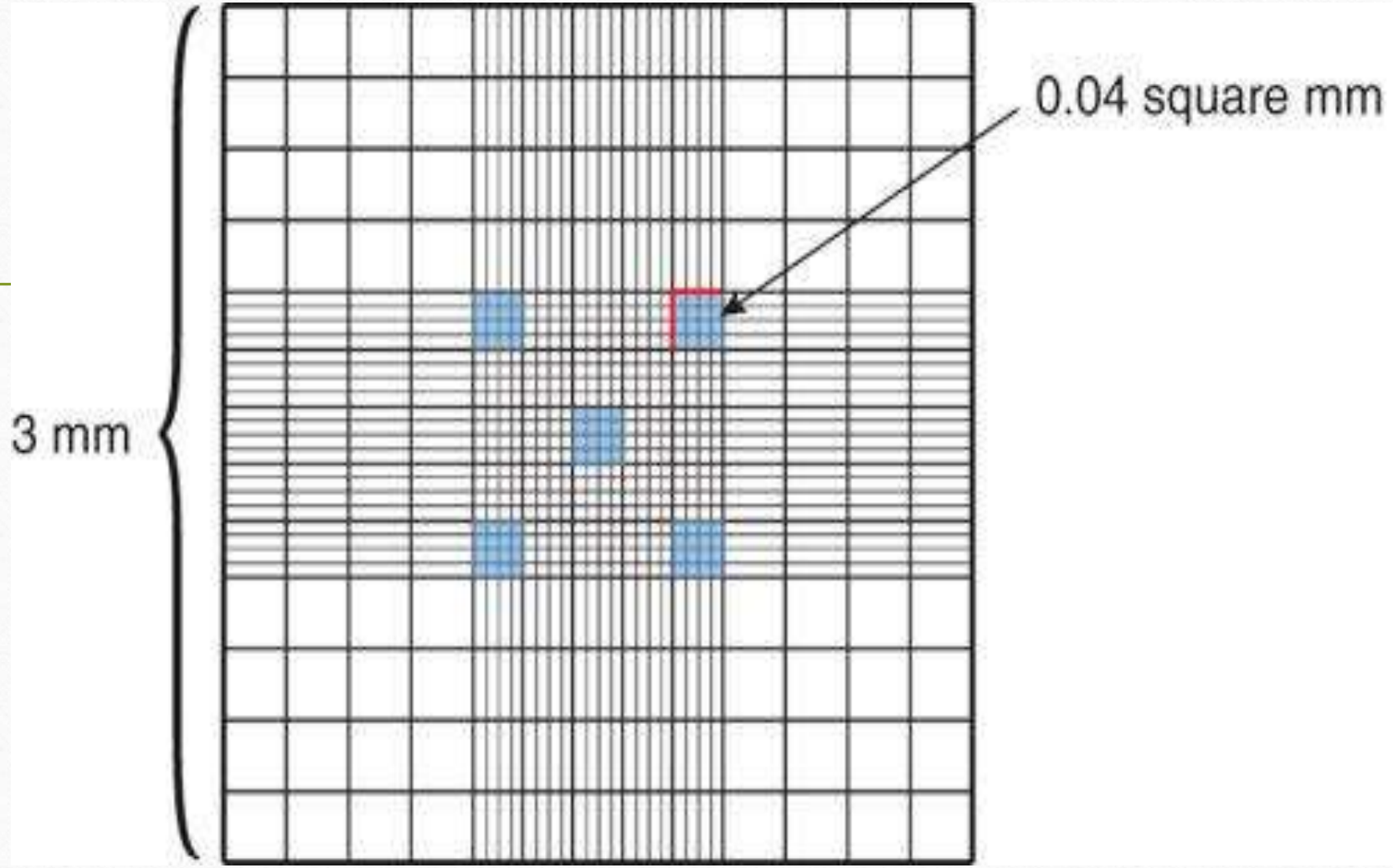


Haemocytometer or improved Neubauer




RAISED RIDGE
SIDE "A"
RULED CHAMBER AREA
SIDE "B"
COVER SLIDE
MOAT
LOADING WELL
CHAMBER DEPTH (0.1 mm)
NEUBAUER RULED COUNTING AREA
3 mm
1 mm
1 mm
1 mm
3 mm
RULED WBC COUNTING AREA
0.2 mm
RULED RBC COUNTING AREA
0.2 mm


0.05
0.2
0.25
1 MM
1 MM
1 MM


0.04 square mm
3 mm

RBC Diluting Fluids

1- Hayem's Solution: ($HgCl_2$, NaCl, Na_2SO_4, H_2O)

- Sodium sulphate (Na_2SO_4) **2.5** gm
- Sodium chloride (NaCl) **0.5** gm
- Mercuric chloride ($HgCl_2$) **0.25** gm
- Distilled water (H_2O) **100** ml

2-Gower's Solution: (Na_2SO_4, Acetic Acid, and H_2O)

- Sodium chloride and sodium sulphate together keeps the **isotonicity of fluid.**
- Sodium sulphate also **prevents clumping of red cells**.
- Mercuric chloride **fixes the cells and acts as a preservative**.

Precautions

1. Counting chamber and pipette should be clean and dry.
2. Fingertip 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. While filling the pipette and charging the counting chamber, no air bubble should enter.
6. Blood should be taken only up to the 0.5 mark and diluting fluid sucked only up to 101 mark.

.

- 7. Blood should be properly mixed with the diluting fluid.
- 8. Discard first few drops before charging because it will not contain RBCs.
- 9. While charging the counting chamber, over filling should be avoided.
- 10. Cells should be settled down and more or less evenly distributed before counting.
- 11. Count from Left to Right and avoid counting of the same cell

Calculation

- * **Dilution factor =** final volume / initial volume = **101-1/0.5=200**
- **Determine+- chamber factor :**

1- area of 1 RBC square **x** depth = volume of 1 RBC square.

0.04 mm^2 x 0.1 mm= 0.004 mm^3.

2- volume of 1 RBC square **x** number of RBC squares counted **=** volume of all squares counted

0.004 mm^3 x 5 = 0.02 mm^3

3- **volume desired/ volume counted = chamber factor**

1/ 0.02= 50

4- chamber factor **x** dilution factor = RBC factor

50 x 200=10000

5- numbers of RBC counted in 5 squares **x** RBC factor = number of RBCs / mm3

- Examples : **500 x 10000 = 5000000 RBCs /mm3**

Calculation

- Cell count (/l) = **N x (D/A) x 10 x 10^6**
- where **N** = total number of cells counted, **D** = dilution of blood, **A** = total area counted (in mm2), **10** = factor to convert area to volume (in µl), assuming a chamber of 0.1 mm depth, and **10^6** = factor to convert count per µl to count per litre.
- Example
- If **2500** cells were seen in the central area (= 1 mm2),

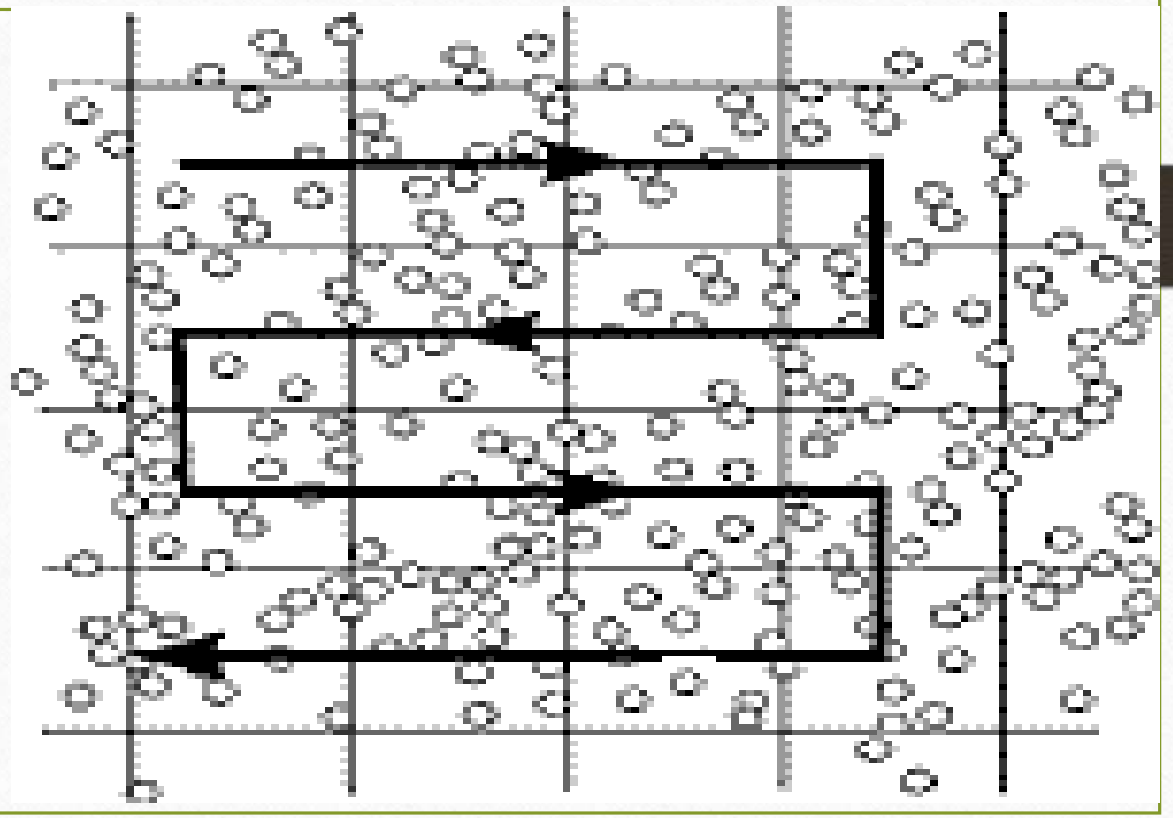
RBC = **2500 x (200/1) x 10 x 10^6 = 500 000 x 10 x 10^6 = 5.00 x 10^{12}/l.**

Errors in hemocytometry most frequently arise as a result of

1. Apparatus
2. Personal technique

Counting Rule

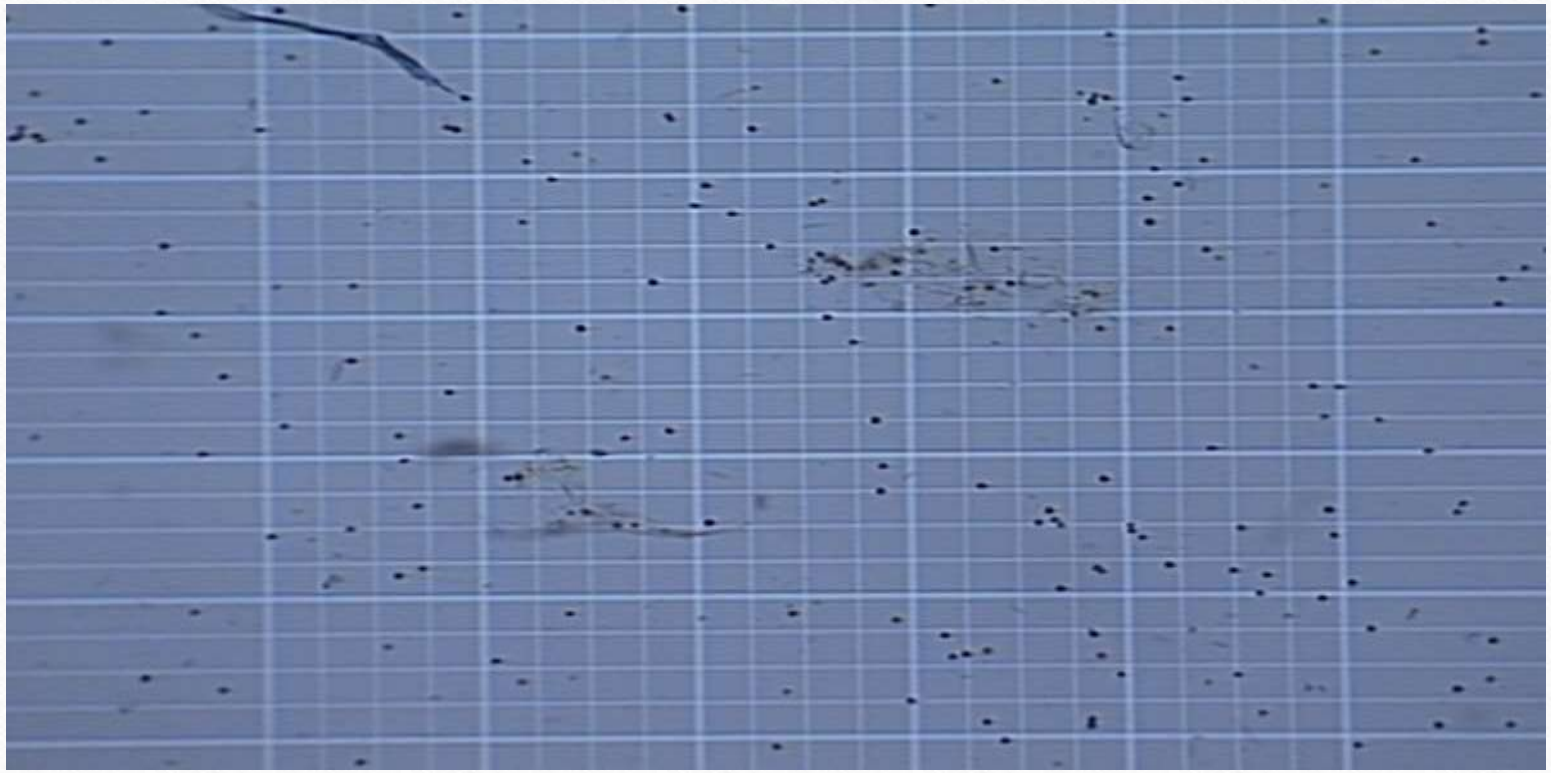
- Do not count cells touching
  - Bottom or upper line
  - Right or left line
- This is to avoid double counting.



FOCUSING

- 4X to see the general formation of slide.
- 10X for WBC counting
- 40X for RBC counting

10x magnification



Home Work

- what is erythropoietin , where is produced , what causes it to produced , and what effect does it have on red blood cell production? (**H.W**)
- Anemia (nutritional , renal , aplastic , hemolytic, megloblastic anemia) (H.W)
- * Polycythemia (H.W)


THE END

Total leukocyte (WBC) Count

White blood cells (WBCs), also called leukocytes, are an important part of the immune system. These cells help fight infections by attacking bacteria, viruses, and germs that invade the body. White blood cells originate in the bone marrow, but circulate throughout the bloodstream.

There are five major types of white blood cells:

- neutrophils
- Lymphocytes (T cells and B cells)
- eosinophil
- monocytes
- basophils

Total leukocyte (WBC) Count test

- This test is almost always a part of **a complete blood count (CBC) test**. A CBC test measures the number of all types of components in the blood, including:

- red blood cells
- white blood cells
- hemoglobin
- hematocrit
- platelets

Definition and Aim

- A WBC count is a blood test used to measure or find out the number of white blood cells (WBCs) in **one cubic millimeter(mm^3)o**f blood.
-

➢ The normal range of WBCs in the blood is

- Newborn 9.0–30.0 x 10^9/L
  9000 – 30 000 /μL

- 2 years 6.2–17.0 x 10^9/L
  6200 – 17000 /μL

- Adult 4.5 - 11 x 10^9 / L
  4,500–10,000 /(μL).

Abnormal results

- A **high** white blood cell count, called **leukocytosis**, may result from a number of conditions and diseases. Some examples include:

- **Infections**, most commonly caused by bacteria and some viruses, less commonly by fungi or parasites.
- **Inflammation** or inflammatory conditions such as rheumatoid arthritis, vasculitis or inflammatory bowel disease.

- Conditions that result in tissue death (**necrosis**) such as trauma, burns, surgery or heart attack.
- **Allergic responses** (e.g., allergies, asthma).
- **Leukemia.**

- A **low** white blood cell count, called **leukopenia**, it is below **4500/µl** can result from conditions such as:

- **Bone marrow damage** (e.g., toxin, chemotherapy, radiation therapy and drugs)
- **Bone marrow disorders**—the bone marrow does not produce sufficient WBCs (vitamin B_{12} or folate deficiency)
- **Lymphoma** (cancer of lymphocyte) or other cancer that has spread (metastasized) to the bone marrow
- **Autoimmune disorders**—the body attacks and destroys its own WBCs (e.g. Systemic lupus erythromatosus)
- **Dietary deficiencies** (deficiency in certain minerals, such as **copper** and **zinc**.)
- Overwhelming infections (e.g., **sepsis**)

- Diseases of the immune system, such as human immunodeficiency virus (**HIV**) or **AIDS** which destroy T- lymphocytes.
- Aplastic anemia and megaloblastic anemia.
- Leukepenia, can also seen in some viral disorders.

When is it ordered?

1. It is performed when an individual undergoes a routine health examination
2. The test may be done when someone has general signs and symptoms of an infection and/or inflammation such as:

- Fever, chills
- Body aches, pain
- Headache

3. when there are signs and symptoms that may be related to a blood disorder, autoimmune disorder, or an immune deficiency.

4. It may also have been ordered periodically for people who take chemotherapy or radiation for cancer treatment.(to monitor the effectiveness of treatment).

HEMOCYTOMETER

- Hemo: blood
- Cyto: cell
- Meter: measurement/counter
- **Thus**, it is an instrument used to count the blood cells.
- *An instrument for counting the blood cells in a measured volume of blood. Also called **a Neubauer's counting chamber**

.

- **It includes:**
  - Neubauer's slide
  - Cover slip

  → Neubauer's chamber (Haemocytometer)
- RBC pipette
- WBC pipette


HAEMOCYTOMETER

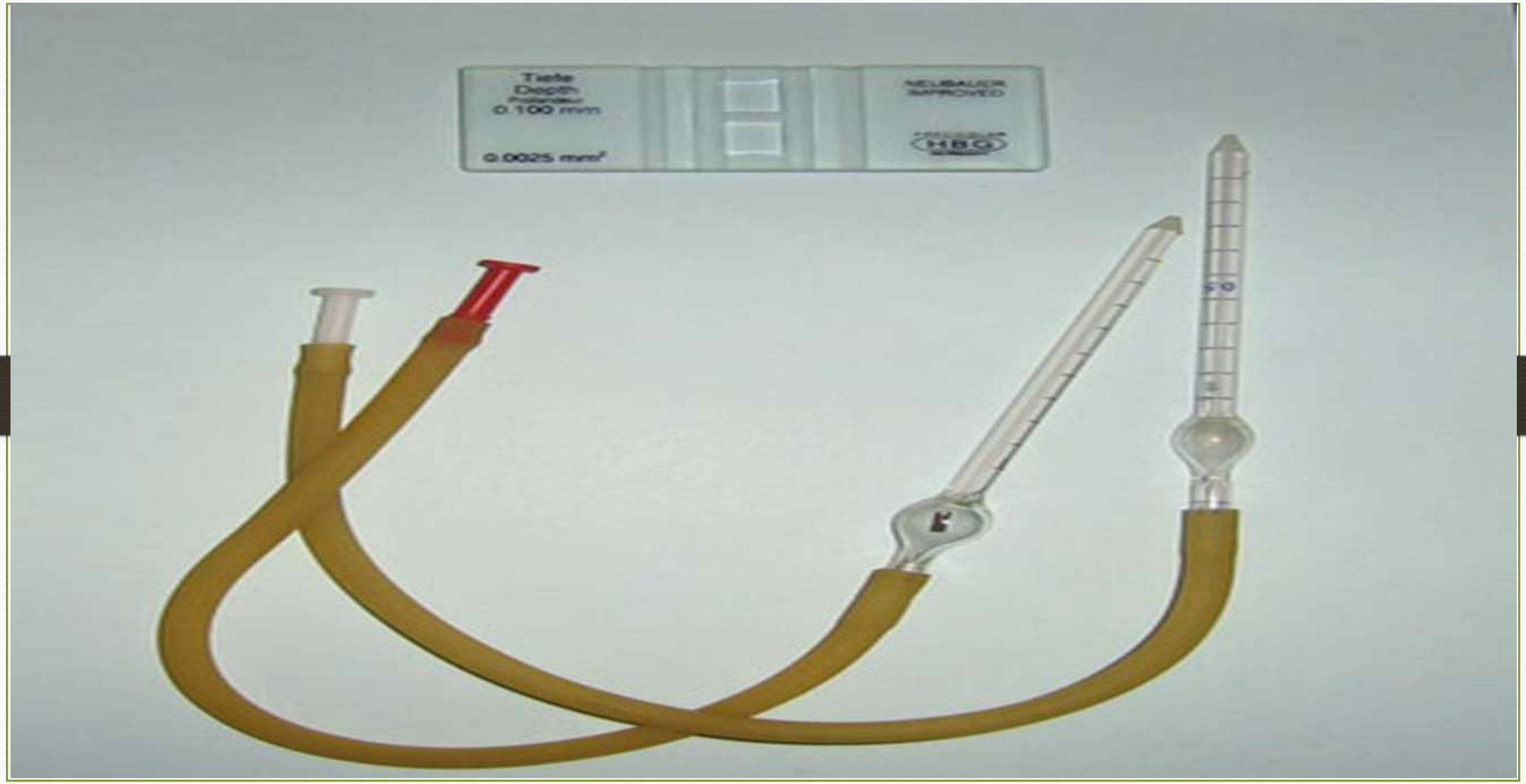
Neubauer's Chamber

- Neubauer's slide with a cover slip over it, is called **a Neubauer's counting chamber**

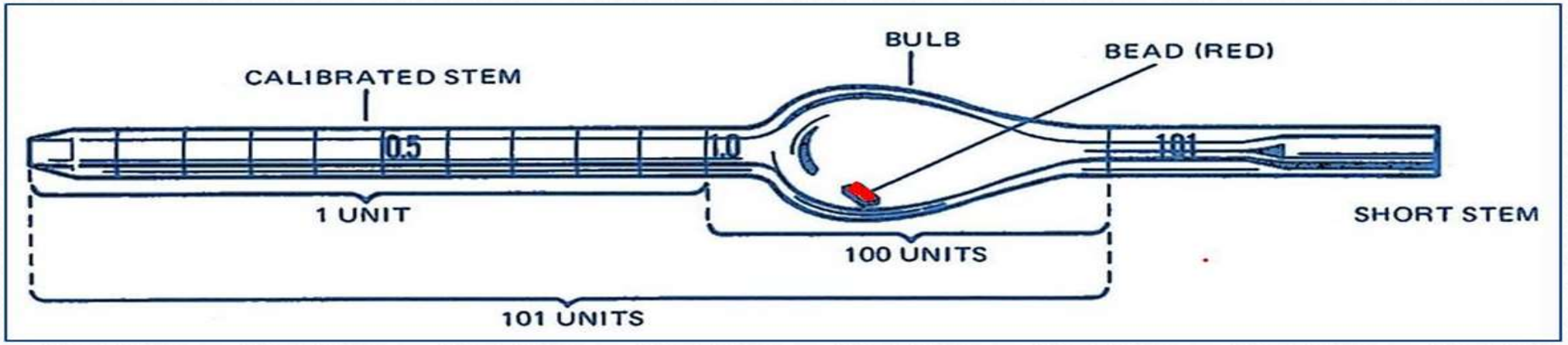
Improved Neubauer Hemocytometer

Differences between RBC and WBC pipette

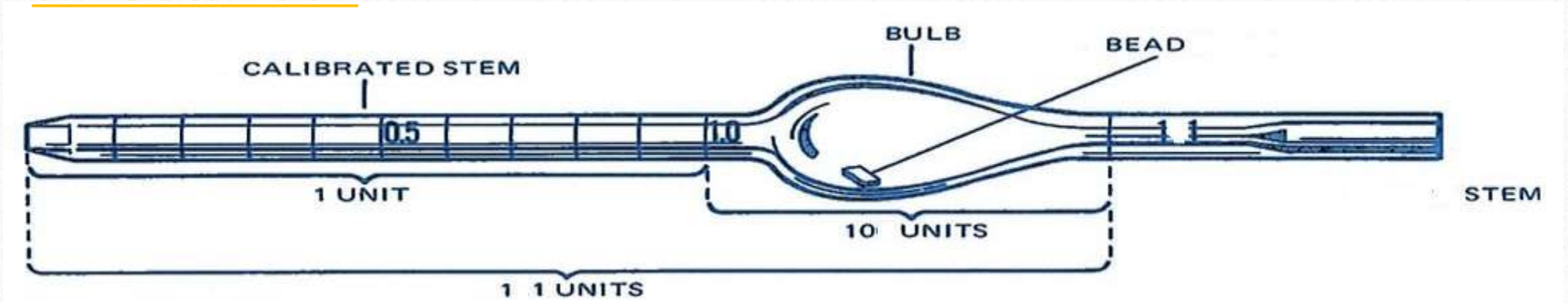
RBC pipette	WBC pipette
1-It has a red bead	1-It has a white bead
2-It has graduations up to mark **101**	2-It has graduations up to mark **11**
3-Size of bulb is larger	3-Size of bulb is smaller


Tiefe
Depth
0.100 mm
0.0025 mm²
NEUBAUER
IMPROVED
HBG

RBC PIPETTE



WBC PIPETTE



PRICIPILE

- The method depends on accurate dilution of a measured quantity of blood using a speacial type of dilution fluid , known as **Turk 's solution** Following adequate mixing, the specimen is introduced into a counting chamber where the white blood cells (leukocytes) in a diluted volume are counted.
-

Reagents and equipment

- 1. Neubauer counting chamber
- 2. Cover slip
- 3. Thoma WBC diluting pipette containing white bead in the bulb.
- 4. Turk's fluid
- 5- microscope
- 6-lancet (is deviced used for capillary blood sampling).
- 7- alcohol

Reagents: White-count diluting fluid. Either of the following diluting fluids may be used:

- (1) **2% of acetic acid** (CH3COOH). Add 2 ml glacial acetic acid to a 100 ml volumetric flask. Dilute and complete the volume to the mark (100) with distilled water.
- (2) **1% of hydrochloric acid** (HCL). Add 1 ml hydrochloric acid to a 100 ml volumetric flask. Dilute and complete the volume with distilled water to 100 ml.
- (3) **Turk 's solution**
- Glacial acetic acid **0.5** ml
- Gentian Violet **1.5** ml
- Distilled water add to **150** ml

Procedure

(1) Draw well-mixed capillary or venous blood exactly to the **0.5** mark in a white blood cell diluting pipette. This blood column must be free of air bubbles.

(2) Wipe the excess blood from the outside of the pipette to avoid transfer of cells to the diluting fluid. Take care not to touch the tip of the pipette with the gauze.

- (3) Immediately draw diluting fluid to the "11" mark while rotating the pipette between the thumb and forefinger to mix the specimen and diluent.
- (4) Mix the contents of the pipette for 3–5 minutes to ensure even distribution of cells. Expel unmixed and relatively cell-free fluid from the capillary portion of the pipette (usually 4 drops).

- (5) Place the forefinger over the top (short end) of the pipette, hold the pipette at a 45 angle, and touch the pipette tip to the junction of the cover glass and the counting chamber.

- (6) Allow the mixture to flow under the cover glass until the chamber is completely charged. Similarly, fill the opposite chamber of the hemacytometer.

- (7) Allow the cells to settle for about 3 minutes. Under low-power magnification and reduced light, focus on the ruled area and observe for even distribution of cells.
- (8) Count the white cells in the four 1 sq mm corner areas corresponding to those marked A, B, C, and D of Figure 1 in each of two chambers.

- (9) Count all the white cells lying within the square and those touching the upper and left -hand center lines. The white cells that touch the right-hand and bottom lines are not to be counted. In each of the four areas, conduct the count as indicated by the "snake-like" line.

➢ **NOTE:** A variation of more than 10 cells between any of the four areas counted or a variation of more than 20 cells between sides of the hemacytometer indicate uneven distribution and require that the procedure be repeated.

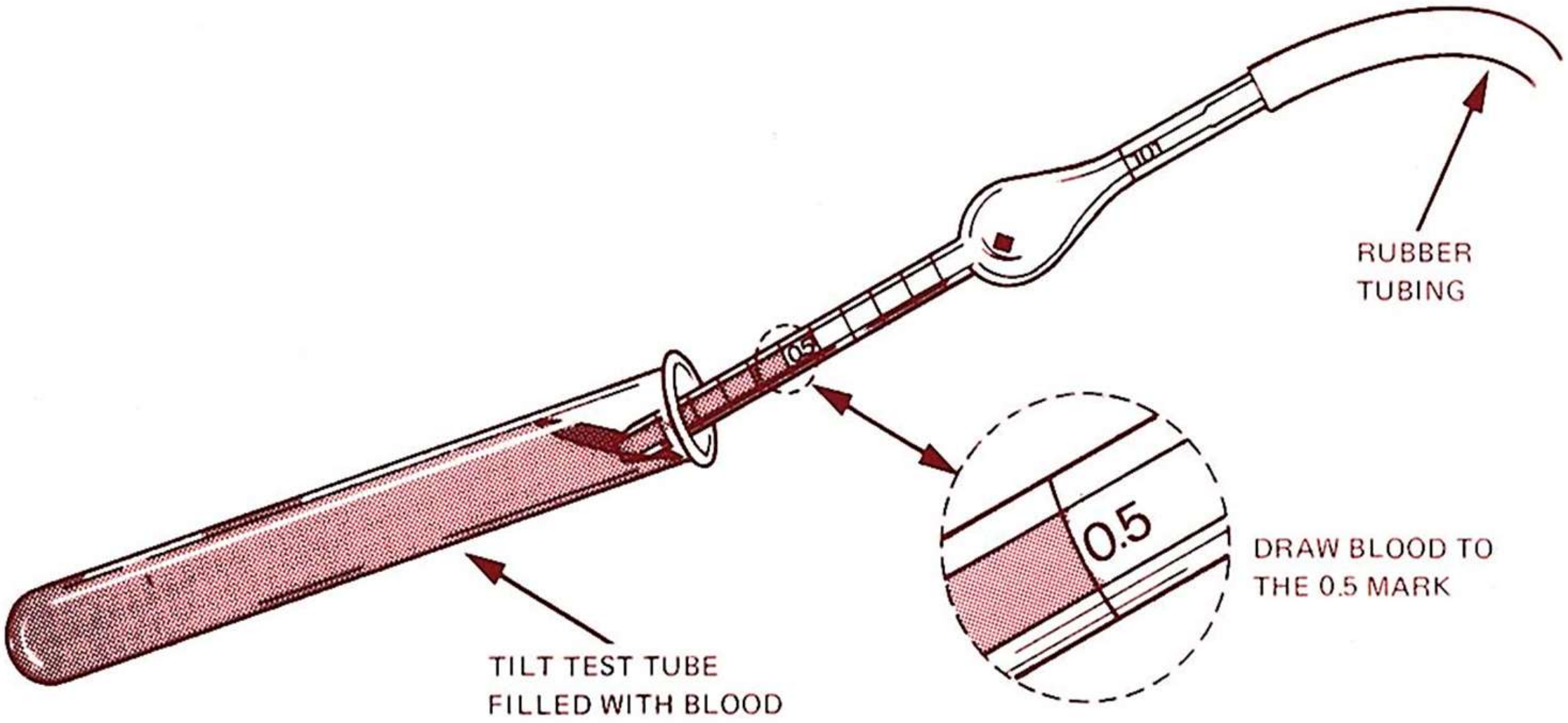
- **NOTE:** If the mixture overflows , or air bubbles occur, clean and dry the chambers, remix the contents of the pipette, and refill both chambers.
- The chamber must be cleaned and the filling process repeated if any of the following defects occur:

1- The fluid overflows

2-The chamber area is not completely filled

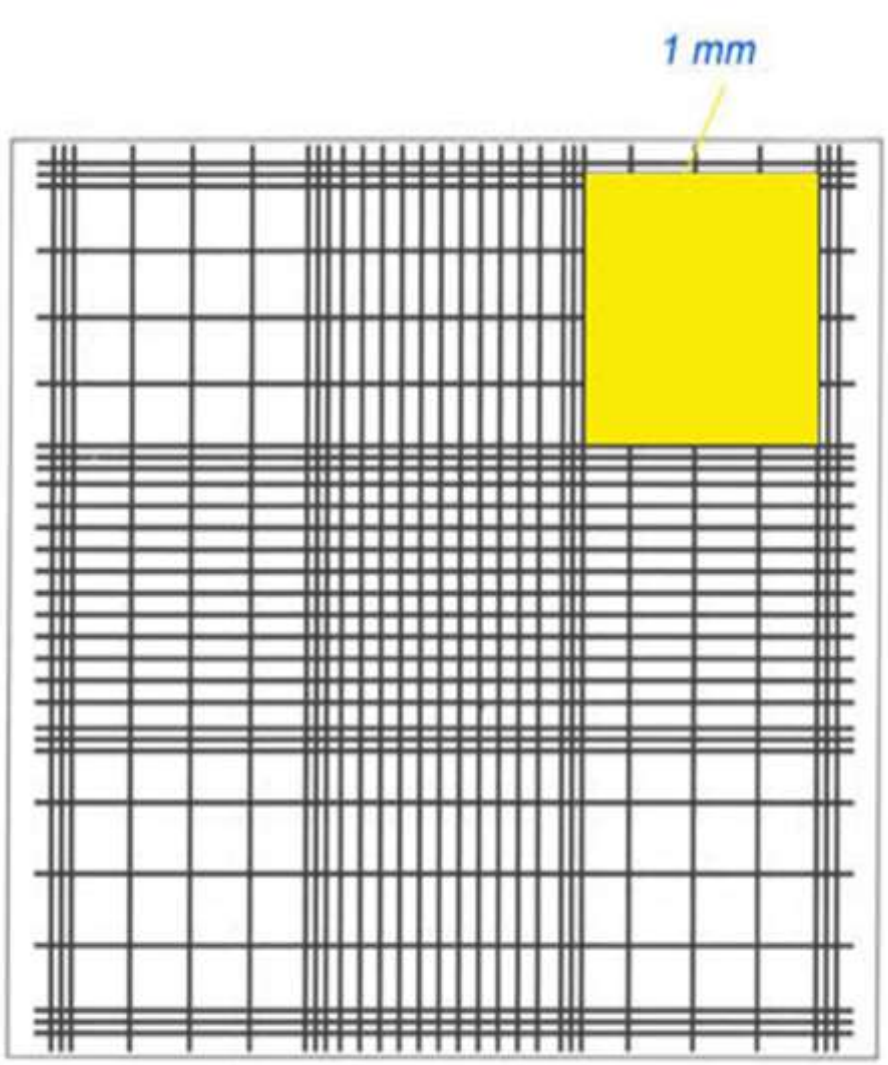
3- Air bubbles occur anywhere in the chamber area

4- Any debris appears in the chamber area

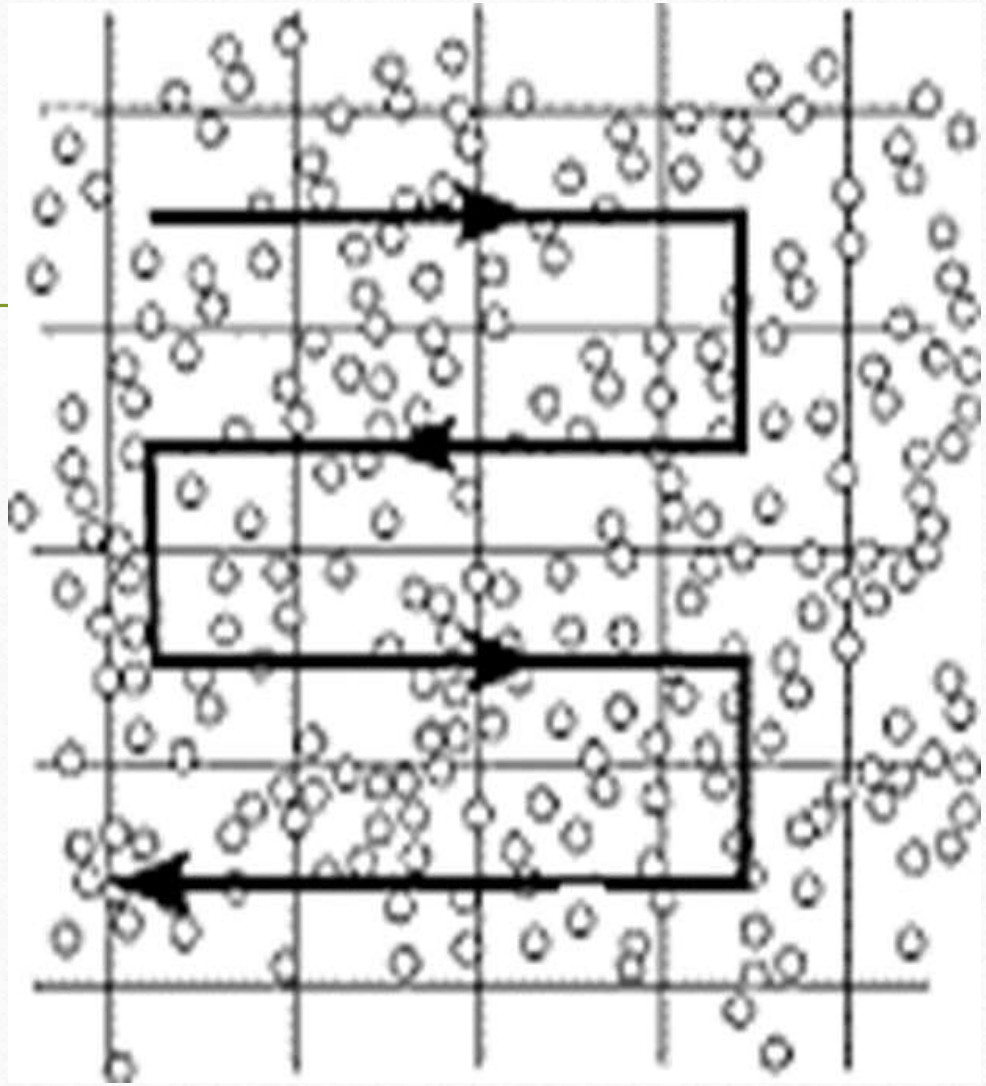

RUBBER
TUBING
0.5
DRAW BLOOD TO
THE 0.5 MARK
TILT TEST TUBE
FILLED WITH BLOOD

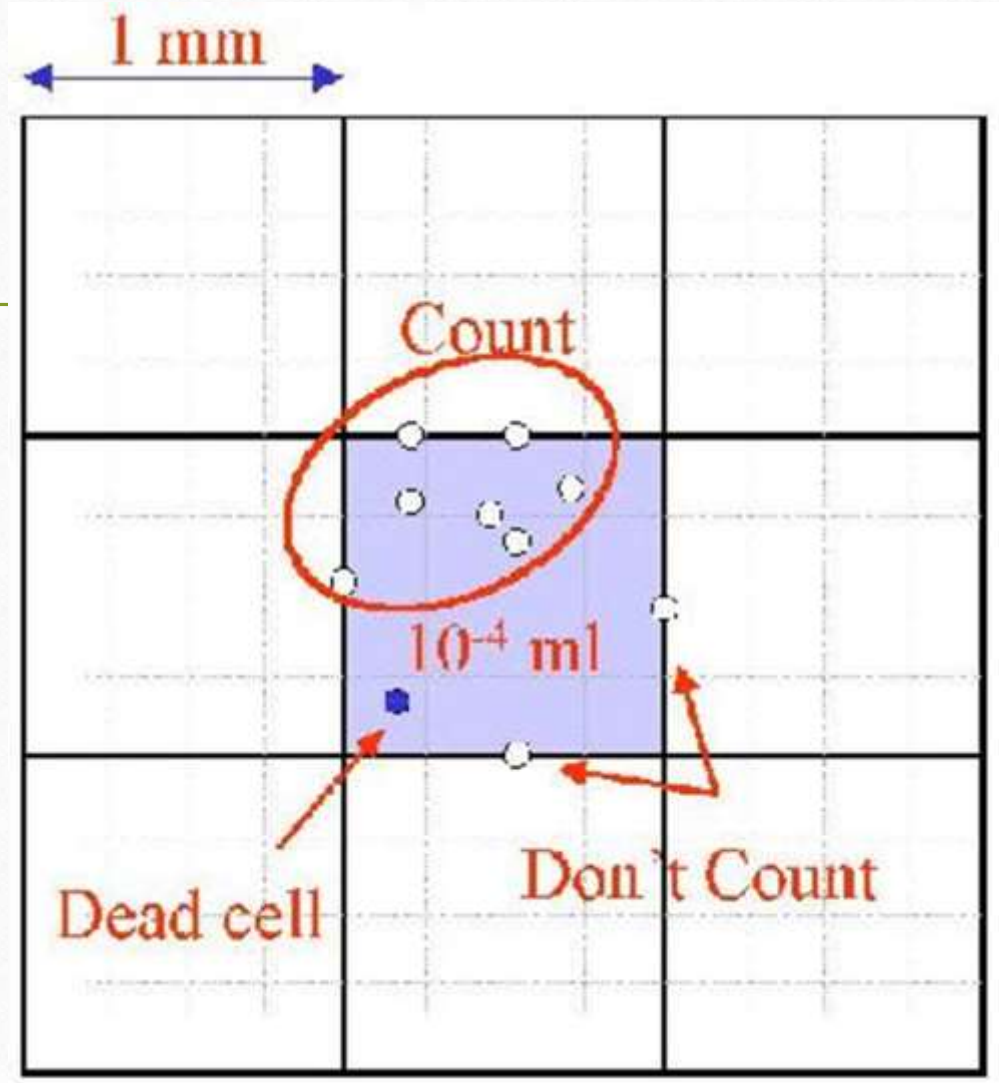
- The four corner squares are further divided into **sixteen** smaller squares and are used for WBC counting.

Four corner squares are meant for WBC counting.
Total = 64 small squares



- Do not count cells touching
- **Bottom or upper** line
- **Right or left** line
- This is to avoid double counting.




1 mm
Count
10^{-4} ml
Dead cell
Don't Count

- **In calculating total count per unit volume of blood 4 important facts must be considered:**

- 1-Total number of cells counted in the four 1mm squares.
- 2-Dilution of the blood sample
- 3-Area counted
- 4-Depth of counting chamber

Calculation

- **(1)** Routinely, blood is drawn to the 0.5 mark and diluted to the 11 mark with WBC diluting fluid. All the blood is washed into the bulb of the pipet (which has a volume of 10). Therefore, 0.5 volumes of blood are contained in 10 volumes of diluting fluid.
- *** Dilution factor = final volume / intial volume = 11-1/0.5=20**

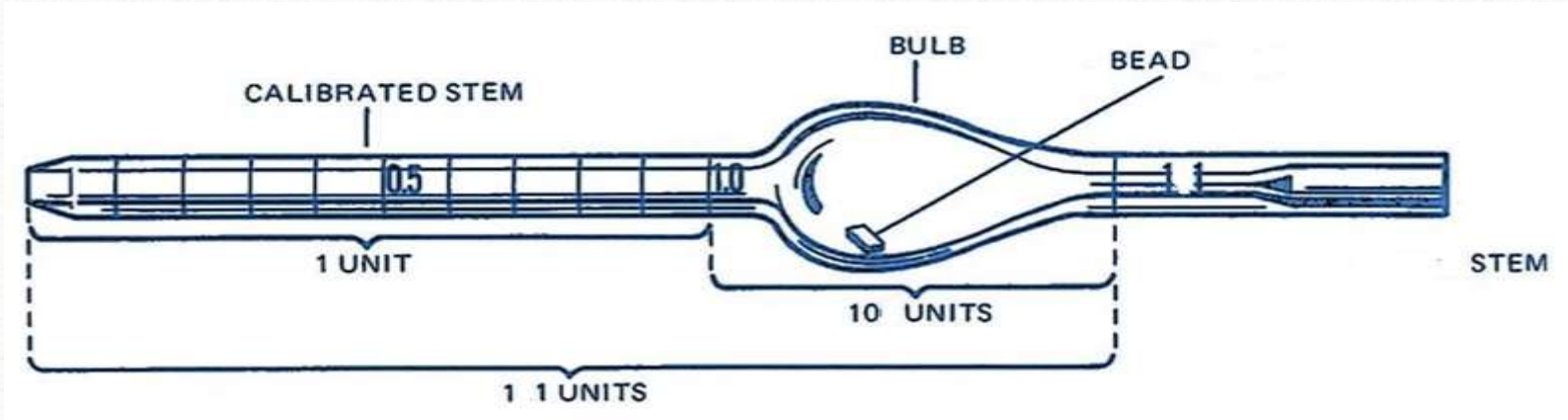
(2) The depth of the counting chamber is **0.1 mm** and the area counted is **4** sq mm (4 squares are counted, each with an area of 1.0 sq mm therefore:

4 x 1.0 sq mm = a total of 4 sq mm).

- ***** The volume counted is: **(area x depth = volume.)**
- **4 sq mm x 0.1 mm = 0.4 cu mm.**

For WBC counting

- **0.5** part of blood is mixed in **10** parts of fluid
  So, **1** part of blood is in **20** parts of fluid
  Thus, dilution factor for WBC counting is **20.**



White cells/µL = cells counted in 4 squares X dilution of blood / volume

➢ **Example :**

- The sum of the cells counted in four 1 seq mm corner areas=140
- 140 x 20 / 4 x 0.1 = 7000 / µL
- 7 X 10 3 / µl X 10 ^{6} = 7 X 10^{9} / L

Calculation

- **Cell count (/l): = N x (D/A) x 10 x 10^6**
- where **N** = total number of cells counted.
- **D** = dilution of blood.
- **A** = total area counted (in mm2).
- **10** = factor to convert area to volume (in µl), assuming a chamber of 0.1 mm depth.
- and **10^6** = factor to convert count per µl to count per litre.

Example

- If **200** cells were seen in the four large corner squares

(= 4 mm^2)

- Count = **200 x (20/4) x 10 x 10^6 = 1000 x 10 x 10^6 = 10.0 x 10^9/L**

Errors in hemocytometry most frequently arise as a result of:

1. Apparatus
2. Personal technique

Errors caused by apparatus:

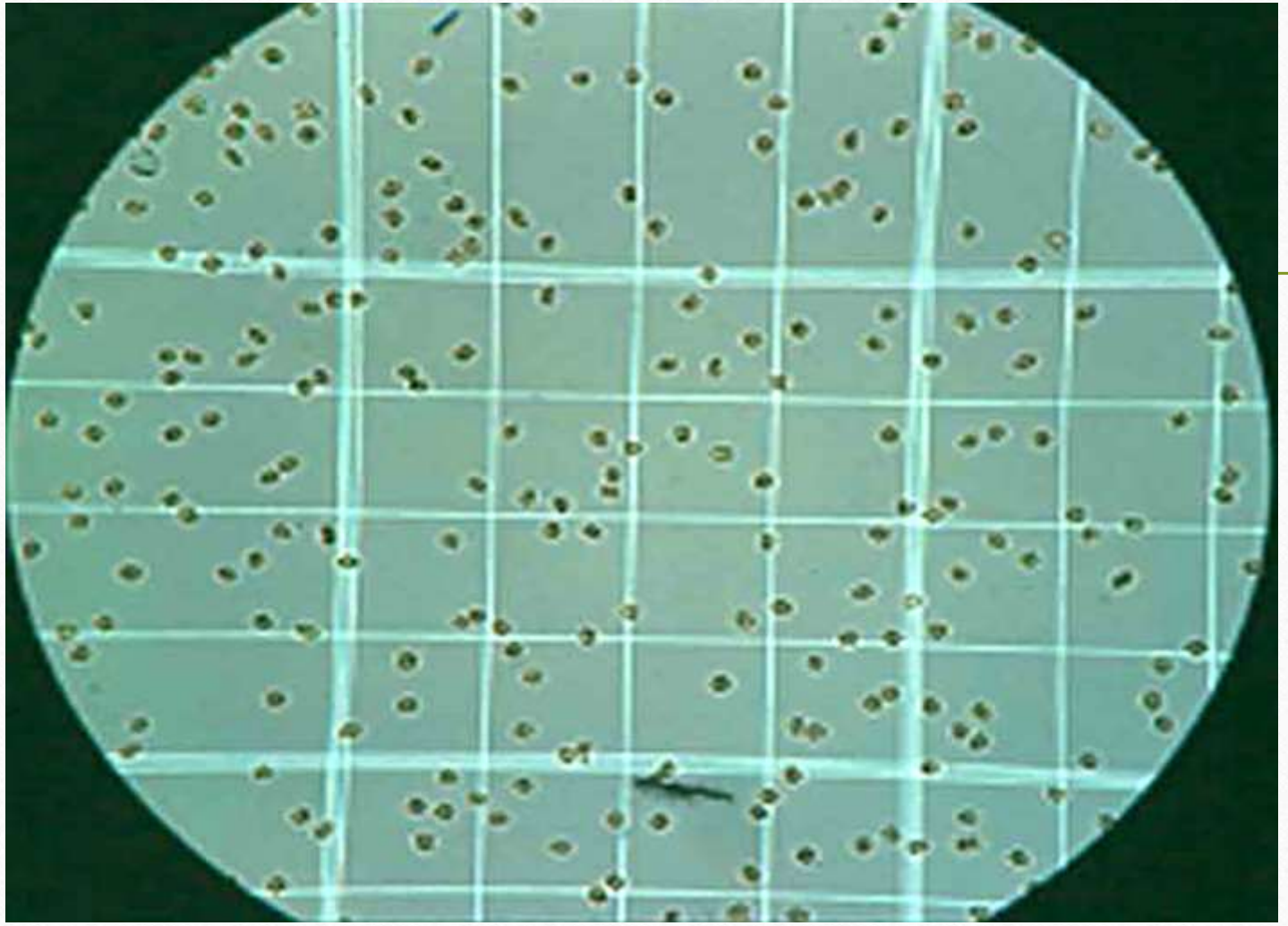
1. chipped pipette tips
2. obscure markings on pipettes
3. dirty glassware
4. inaccurate rulings on chamber

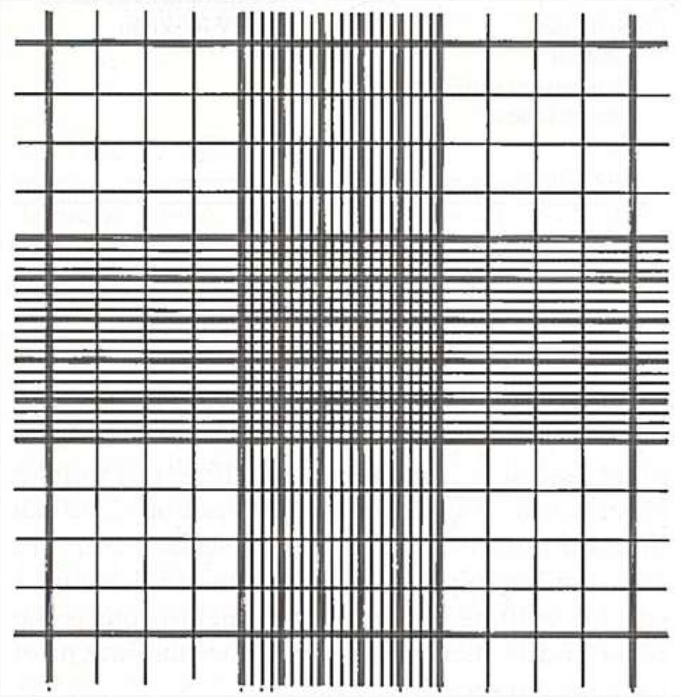
Errors caused by personal technique:

1. Not thoroughly mixing blood
2. Failure to discard first 4 drops
3. Not loading chamber properly (overfilling, trapped air bubbles)
4. Counting cells inaccurately (counting cells twice, counting on wrong borders)
5. Calculation error

FOCUSING

- 4X to see the general formation of slide.
- 10X for WBC counting
- 40X for RBC counting





Calculation and discussion (H.W)

First Chamber Cells counted in each square	Second Chamber Cells counted in each square
35	45
40	36
44	37
49	44
158 WBCs counted	162 WBCs counted


THANK
YOU