
بسم الله الرحمن الرحيم

BLOOD MODULE

FIRST-YEAR MBBS BATCH 51 (2024)

SKILL LAB /Physiology PRACTICAL

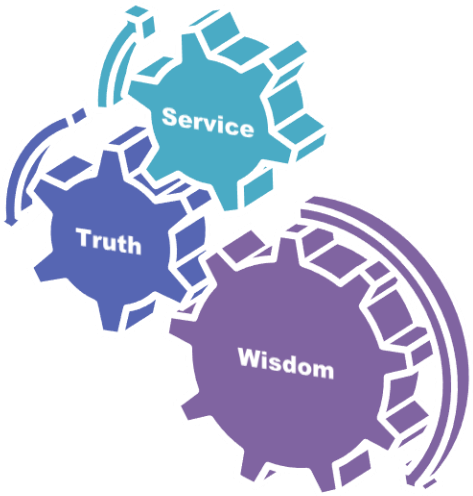
Introduction to Wintrobe and Westergren Tube

Usman

Date: 25-01-2024
to
00-00-0000

Table of contents

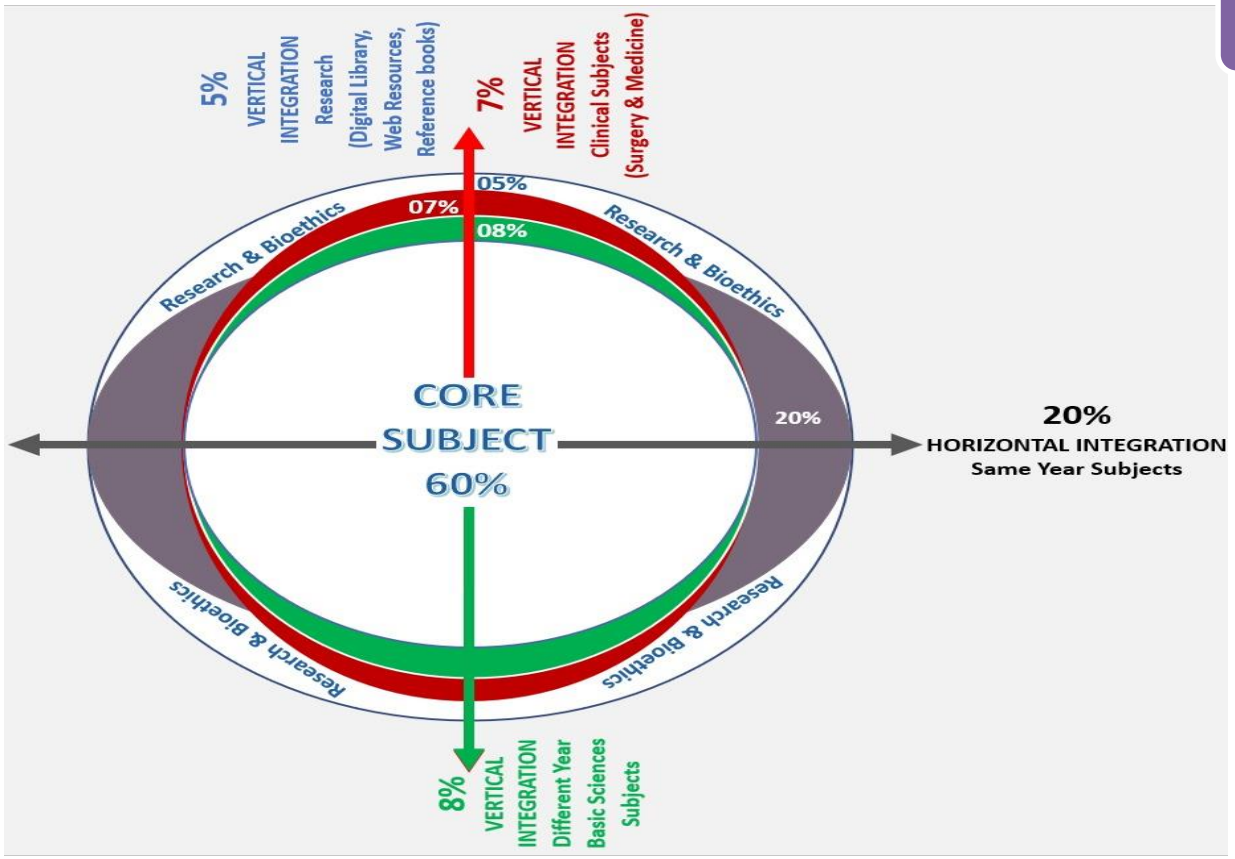
Motto



Vision; The Dream/Tomorrow

- To impart evidence-based research-oriented medical education
- To provide the best possible patient care
- To inculcate the values of mutual respect and ethical practice of medicine

Professor Umar Model of Integrated Lecture



60% CORE SUBJECT

20% HORIZONTAL INTEGRATION Physiology biochemistry

8% VERTICAL INTEGRATION Pathology pharmacology

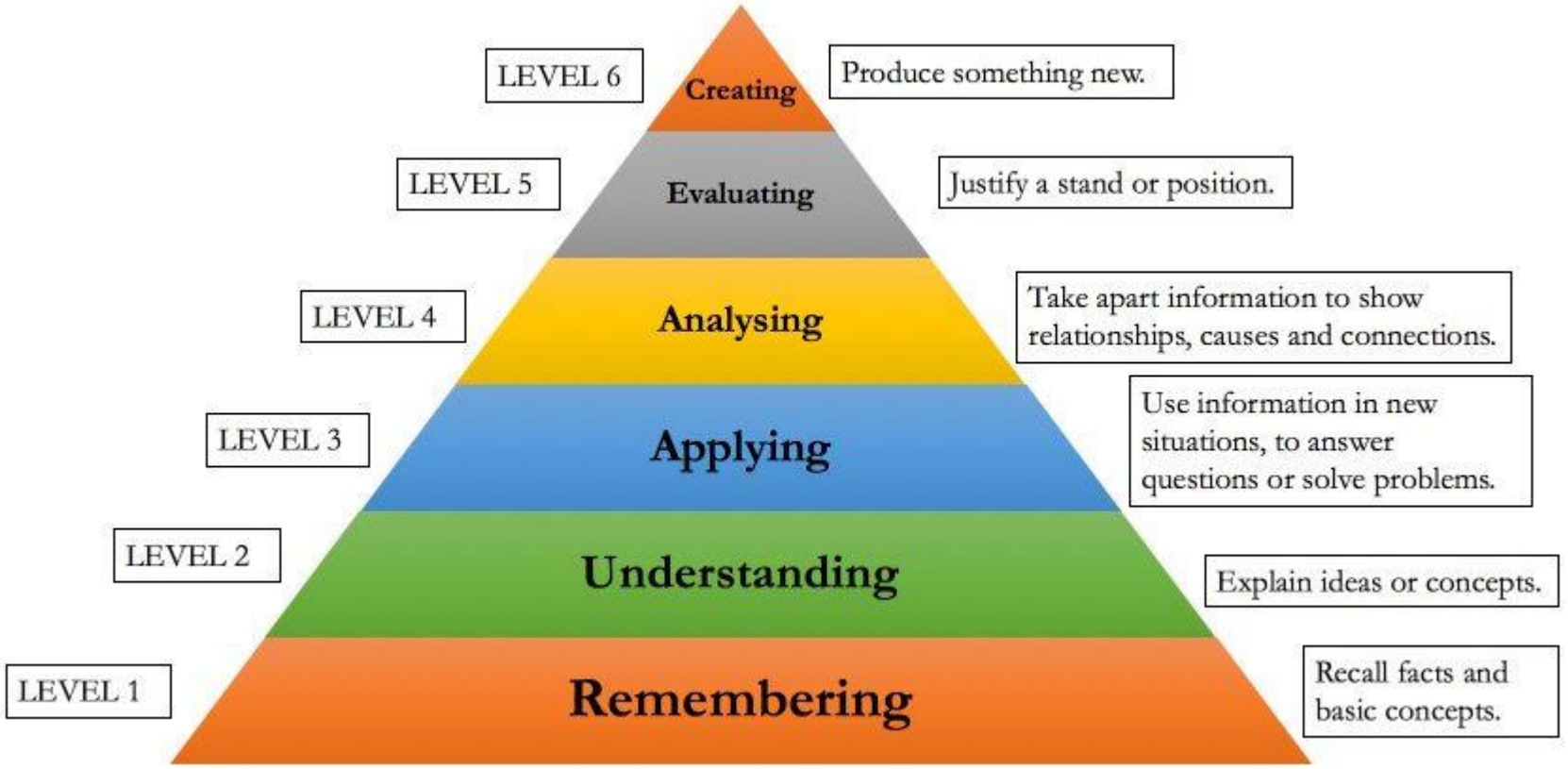
7% VERTICAL INTEGRATION Clinical integration

5% VERTICAL INTEGRATION Research, professionalism Ethics Digital library

Bloom's Taxonomy : Domains Of Learning

Sr. #	Domain of learning	Abbreviation	Levels of the domain	Meaning
1	cognition	C	C1	Recall / Remembering
2			C2	Understanding
3			C3	Applying / Problem solving
4	Psychomotor	P	P1	Imitation / copying
5			P2	Manipulation / Follows instructions
6			P3	Precision / Can perform accurately
7	Attitude	A	A1	Receiving / Learning
8			A2	Respond / Starts responding to the learned attitude
9			A3	Valuing / starts behaving according to the learned attitude

Bloom's Taxonomy Of The Cognitive Domain



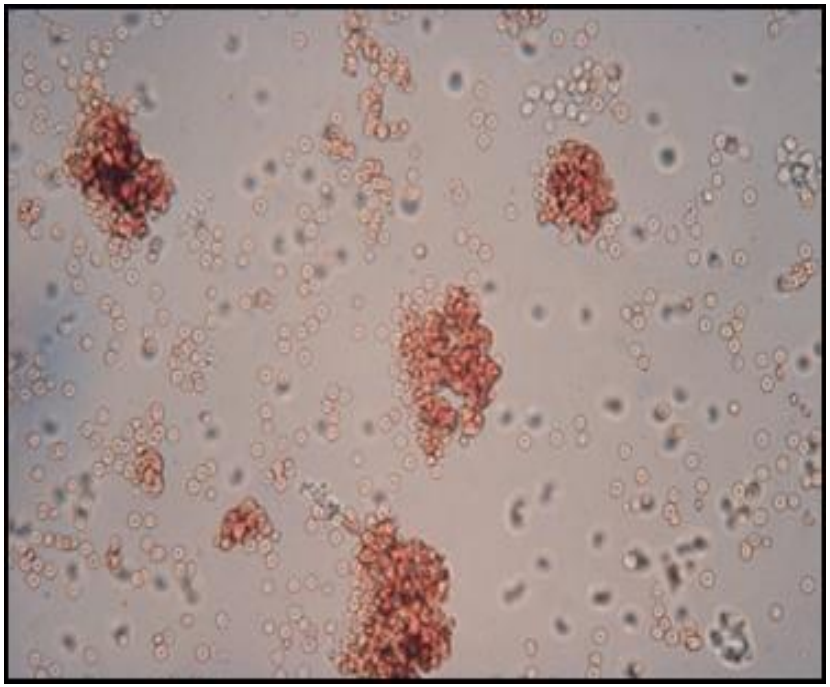
Learning Objectives

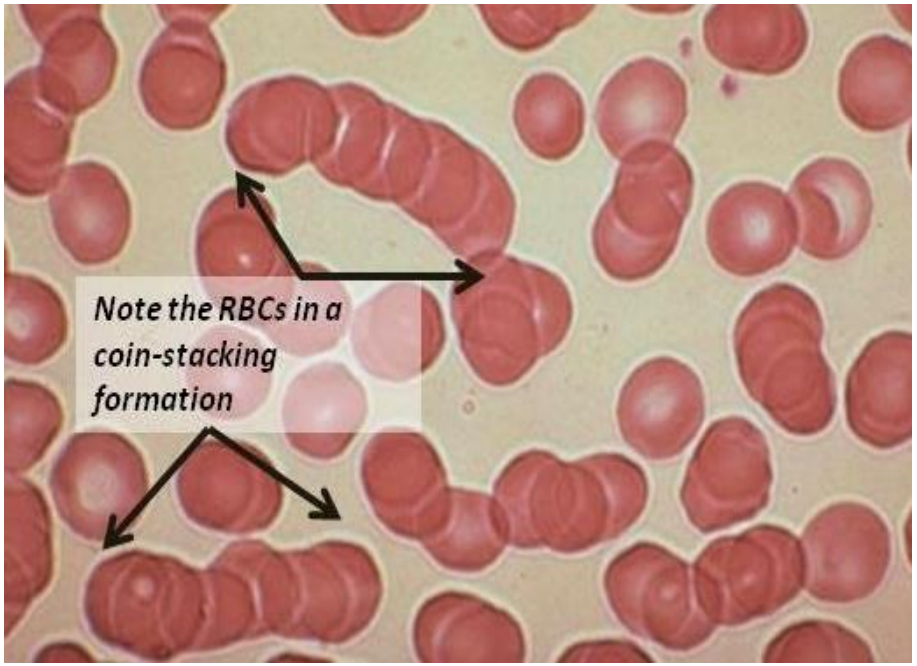
Sr. #	Learning Objective	Domain of Learning
1	To describe the physiological basis of ESR and its clinical significance.	C1
2	To discuss the advantages and disadvantages of the ESR determination by Westergren and Wintrobe methods	C2
3	To perform ESR measurement on a sample using the Westergren and Wintrobe methods.	C1
4	To apply knowledge of ESR measurement in a clinical context to assist in making diagnostic decisions	A3, P3, C3

Horizontal Integration With Histology

Autoagglutination vs Rouleaux Formation

Rouleaux formation is the linking of RBCs into chains resembling stacks of coins.





Core Concept

Introduction

i. Definition

- Erythrocyte sedimentation rate is the rate of fall (sedimentation) of red cells when anticoagulated blood is allowed to stand undisturbed for a specified period, usually 1 hour.
- Expressed in mm/hr.

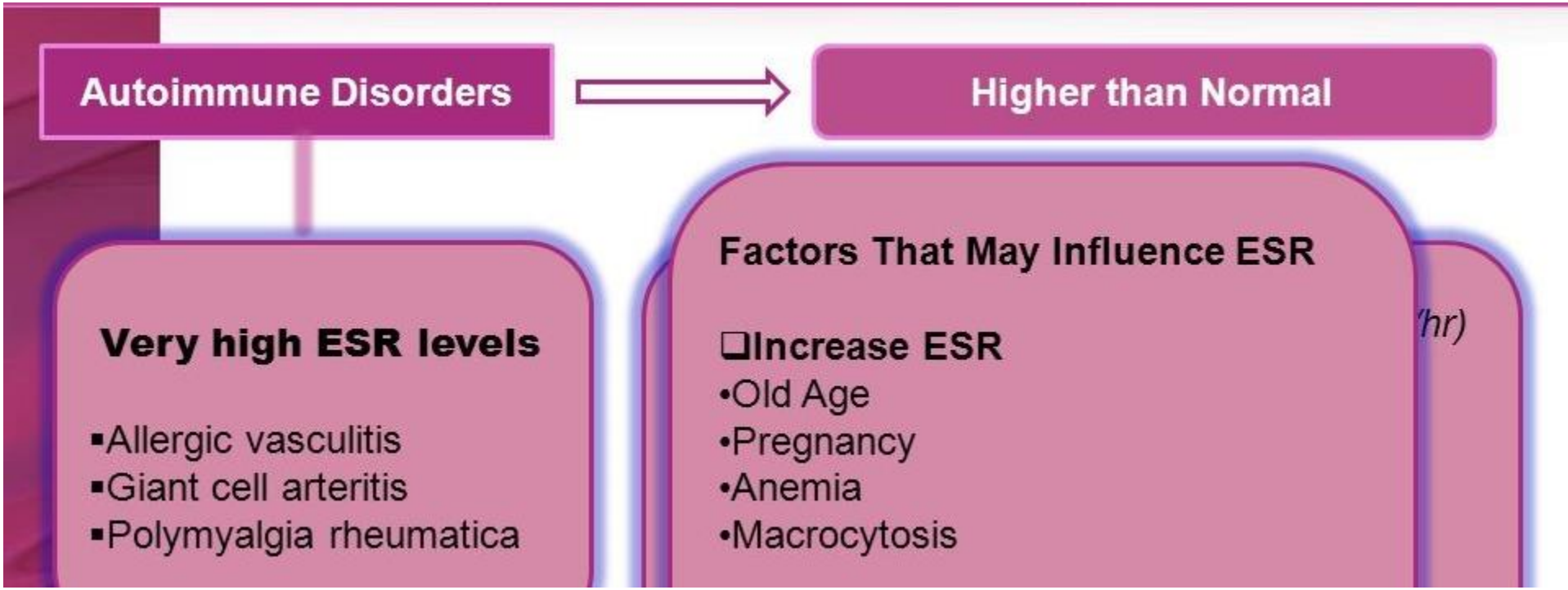
Introduction

- A nonspecific test
- Used as an index of the presence and extent of inflammation (the so-called 'acute phase response') and its response to treatment, e.g., tuberculosis, and rheumatoid arthritis.

Clinical Significance

Raised in:

- Majority of acute or chronic infections
- Most neoplastic and degenerative diseases



Principle

- The ESR is determined by filling a narrow pipette of predetermined length and bore, with well-mixed anticoagulated blood and placing it in a vertical position for a set time at the end of which the distance from the top of the column to the interface between the plasma and the sedimented red cells is recorded and expressed in **mm/unit** time.

Stages in ESR

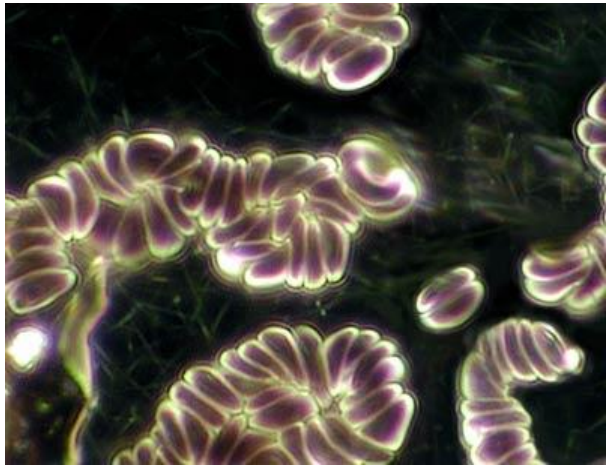
ESR has three stages:

i. An initial period of 10 minutes → **rouleaux** formation takes place

ii. A period of approximately 40 minutes → settling or **sedimentation** occurs at a constant rate, and

iii. A slower rate of fall (last 10 minutes)→ **packing** of the sedimented red cell column occurs.

* The second stage is the most significant phase.



RBCs Rouleaux

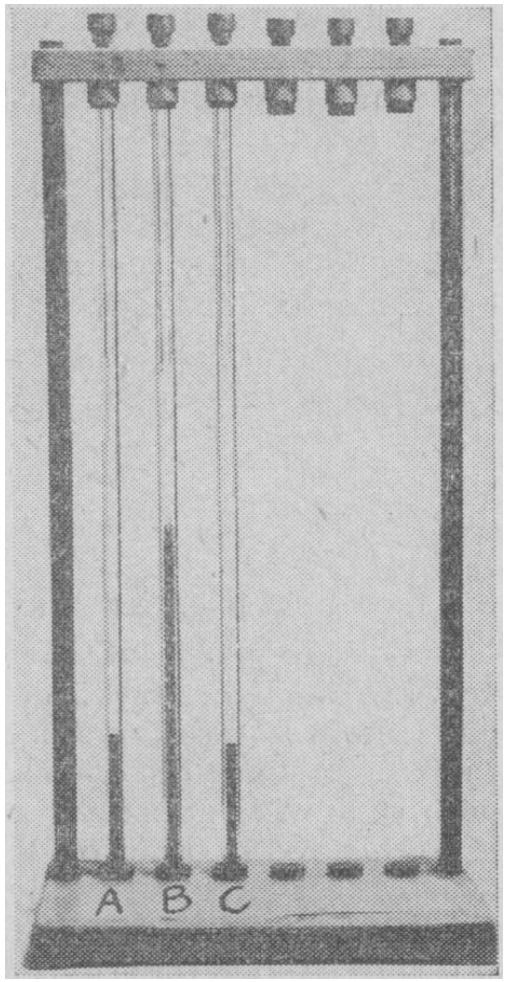
Determination of ESR

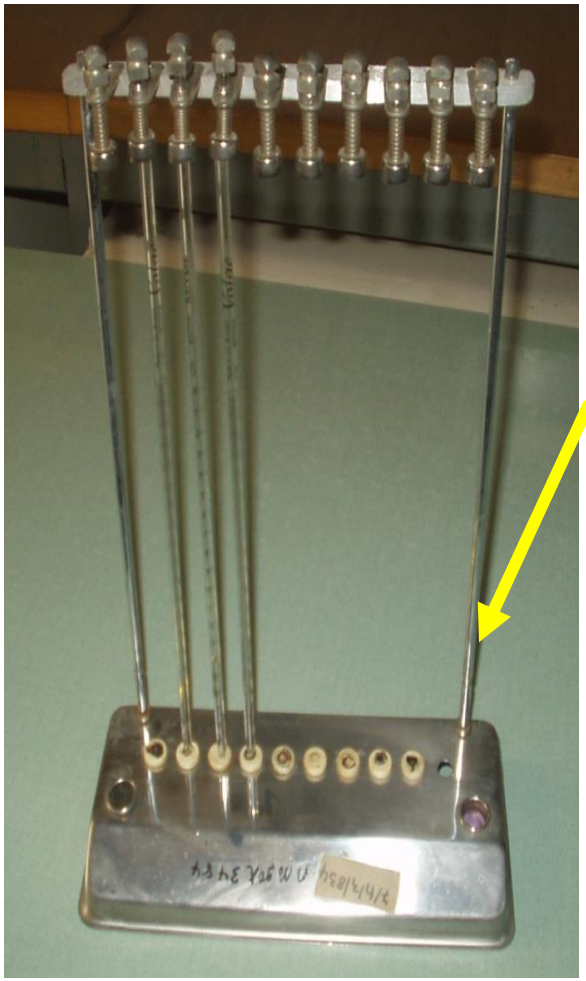
Two basic methods

1. Westergren Method
2. Wintrobe method

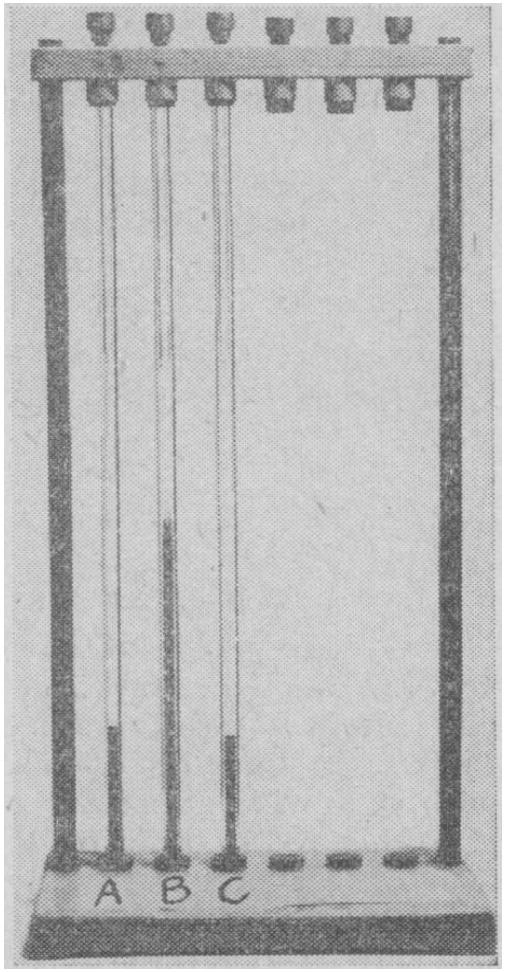
The Westergren Method

- This is the ICSH reference method for ESR determination.





Westergren rack or stand with an open glass tube



Westergreen rack & tube filled with blood sample

Procedure

1. Venous blood is diluted accurately in the proportion of one volume of citrate to four volumes of blood.
   - ✓ The blood may be directly collected into the citrate solution or an EDTA anticoagulated blood used.
   - ✓ Mix thoroughly by gentle repeated inversion.
   - ✓ ESR preparations should preferably be set up within 2 hrs of blood collection, but under extenuating circumstances may be refrigerated overnight at 4oC before testing.
2. A clean dry Westergren-Katz pipette is carefully filled and adjusted to the "0" mark on top.

Westergren method Cont'd..

3. The pipet is placed in a strictly vertical position in the Westergren stand
   - under room temperature conditions
   - not exposed to direct sunlight and
   - away from vibrations and draughts
4. Allow it to stand for exactly 1 hour
5. After 1 hour read to the nearest 1mm the height of the clear plasma above the upper limit of the column of sedimenting red cells.

Westergren method Cont'd..

Reporting

- ✓ The result is expressed as ESR = X mm/hr

- ✓ * A poor delineation of the upper layer of red cells, the so-called ***'stratified sedimentation'***, has been attributed to the presence of many reticulocytes.

Westergren method Cont'd..

Advantages

- It more reliably reflects the clinical state
- is the most sensitive method for serial study of chronic diseases, e.g., tuberculosis.

Disadvantages

- Requires a large amount of blood.
- Involves dilution which may be one source of error.

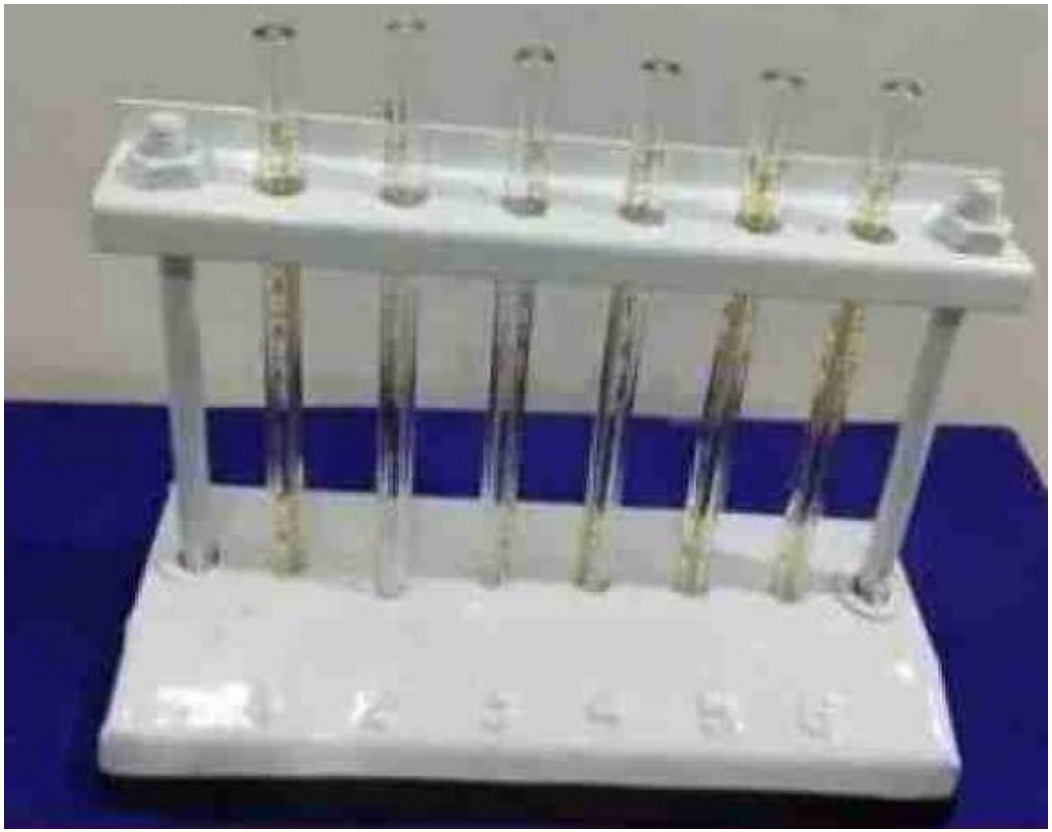
Normal Range:

Men: 0-15mm/hr

Women: 0-20mm/hr

The Wintrobe Method

- Utilizes a tube closed at one end
- Tube has a length of 11cm
- Bore diameter of the tube is 2.5mm
- Features a graduated scale ranging from 0-100mm
- Requires a special Wintrobe rack for the ESR determination process.



Wintrobe rack

Procedure

1. Blood is collected with EDTA in the right proportion.
2. Enough blood to fill the Wintrobe tube (approximately 1ml) is drawn into a Pasteur pipette having a long stem.
3. The Wintrobe tube is then filled from the bottom up (so as to exclude any air -bubbles) to the "0" mark.
4. The tube is placed in the Wintrobe rack in exactly vertical position and the time is noted.
5. At the end of 1hour the ESR is read as the length of the plasma column above the cells and is expressed as x mm/hr.

Wintrobe method cont'd

Advantages

- The method is:
  - simple
  - requires a small amount of blood
  - no dilution required
- With the same preparation, once the ESR has been read, the hematocrit value can be determined after centrifugation.
- Microbilirubin determination can be made on supernatant plasma and smears of buffy coat can be made.

Wintrobe method (cont'd)

Disadvantages

- Because of the short column, it is only sensitive when the ESR is low and when the disease is in the acute stage.
- **Normal Range**
  - Men: 0-7mm/hr
  - Women: 0-15mm/hr

Quality control

- Strictly adhere to SOP (timing, positioning the ESR rack, etc)!
- Quality control samples are commercially available

Sources of error

- Improper filling of tubes
- Old specimen (should be performed within 2 hours of collection)
- Cold agglutinins can cause a falsely elevated ESR
- Clotted and hemolysed samples

Technical tips

- Tube must be completely filled to the zero mark
- Hemolyzed specimen is not accepted
- There should be no air bubble
- Refrigerated specimens must come to room temperature for 30 minutes prior to testing
  - refrigerated sample is used within 24 hours, if the test can not be performed within 2 hours of sample collection
- The ESR rack must be on a level surface and free of vibration
- Strictly follow SOP

Vertical integration with Clinical & Paraclinical Sciences

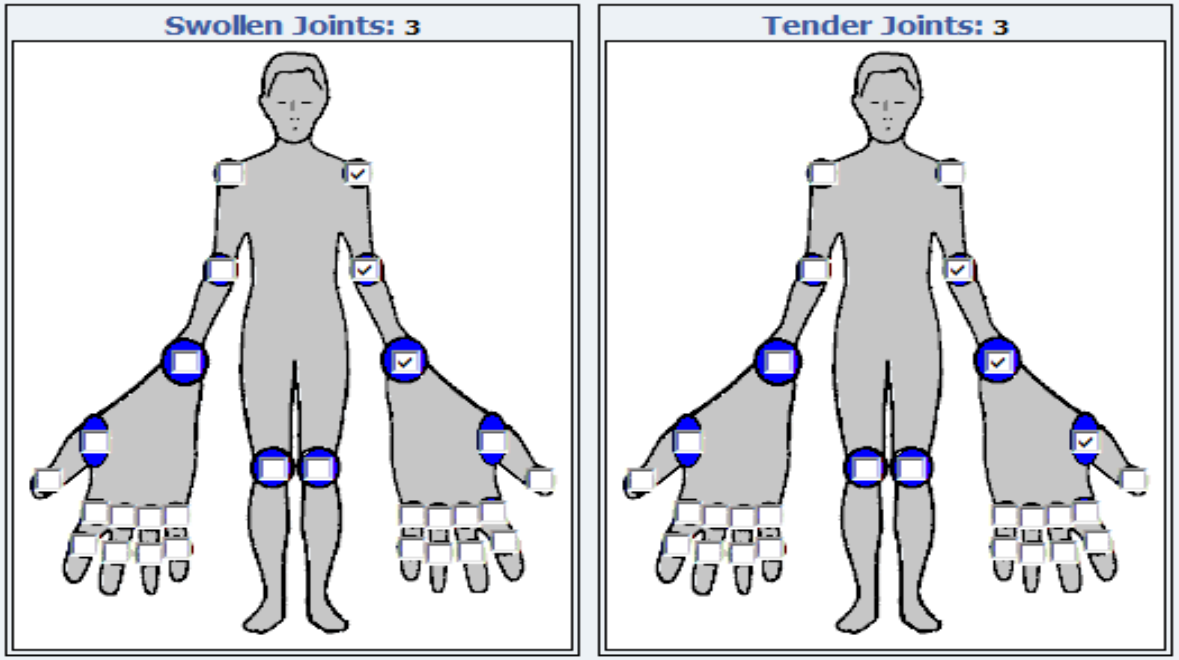
ESR & C-reactive Protein Measurements and Their Relevance in Clinical Medicine

High ESR/Low CRP	High CRP/Low ESR
• Infections (Bone and joint)	• Infections (urinary tract, gastrointestinal tract, lung and bloodstream)
• Connective tissue disease (SLE)	• Myocardial infarction
• Ischemic stroke	• Venothromboembolic disease
• Malignancy	• Rheumatoid arthritis
• Renal insufficiency	• Low serum albumin
• Low serum albumin	

Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; SLE, systemic lupus erythematosus.

DAS28-ESR for Rheumatoid Arthritis

Patient Name:	Oliver Hardy	**DOB:**	01/04/1970
Unit Number:	H65-432-1	**Sex:**	M
Date recorded:	05/04/2013		
	ESR 11.0 CRP 9.0	**Das28 Result ESR**	**4.17** High
Patient disease activity: *(visual acuity scale, VAS, 0-100mm)*	74 (0 - not active 100 - extremely active)	**Das28 Result CRP**	**4.28** High



Preferred calculation ESR

FORMULAE:
DAS28-ESR *(4)* = 0.56*sqrt(TJC28) + 0.28*sqrt(SJC28) + 0.70*Ln(ESR) + 0.014*VAS
DAS28-CRP *(4)* = 0.56*sqrt(TJC28) + 0.28*sqrt(SJC28) + 0.36*ln(CRP+1) + 0.014*VAS + 0.96

http://www.das-score.nl/

Biomedical Ethics

4 PILLARS OF MEDICAL ETHICS

THE PILLAR OF BENEFICENCE

THE PILLAR OF NON-MALEFICENCE

THE PILLAR OF AUTONOMY

THE PILLAR OF JUSTICE

Non-maleficence (Lesson of the day)

- The principle of non-maleficence holds that there is an obligation not to inflict harm on others.
- Example: stopping a medication known to be harmful or refusing to give a medication to a patient if it has not been proven to be effective.

Brain Storming

Question & Answer

QUESTIONS

QUESTION 1:-

Define the ESR.

Ans: The Erythrocyte Sedimentation Rate (ESR) is a blood test that gauges the speed at which red blood cells settle, serving as a general marker for inflammation in the body and aiding in the detection and monitoring of various inflammatory conditions.

QUESTIONS

Question 2: List the items required in ESR determination using the Westergren method.

Answer

I. Blood Sample (collected in an EDTA tube)
II. Westergren ESR Tube
III. Rack or Stand
IV. Timer or Stopwatch
V. Laboratory Pipette
VI. Mixing Device
VII. Paper or Plastic Capillary Stopper
VIII. Clean Cloth or Tissue
IX. Incubator or Room Temperature Control (if needed)
X. Refrigerator (if needed)

QUESTIONS

Question 3: List at least five sources of error and their remedies in ESR determination

Answer

I. Incorrect blood-to-anticoagulant ratio
II. Inadequate mixing of blood
III. Incorrect reading time
IV. Temperature fluctuations
V. Improper tube handling

Suggested Research Article

Related Research Article

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Abstract

The erythrocyte sedimentation rate (ESR) is a routine test for inflammation. Few studies have investigated the potential influence of lifestyle factors and common metabolic abnormalities on the ESR. This study investigates the influence of demographic factors, alcohol consumption, smoking, physical activity, obesity, and metabolic syndrome on the ESR in adults.

This cross-sectional study covered 1472 individuals (44.5% males; age range, 18–91 years) randomly selected from the population of a Spanish municipality. The ESR was measured using a standardized method. We assessed habitual alcohol consumption in standard drinking units, along with tobacco smoking, regular physical exercise (by questionnaire), body mass index, and variables defining metabolic syndrome. Multivariate analyses were performed, including mean corpuscular volume and hemoglobin concentration in the models.

The ESR was higher in females than in males, and increased steadily with age. Median ESR of females was 2-fold higher than that of males, and median ESR of individuals aged >65 years was 2-fold higher than that of individuals in the youngest category (ages 18–35 years). Body mass index, presence of metabolic syndrome, and smoking were independently and positively associated with higher ESR values. Light alcohol drinkers and individuals with high regular physical activity displayed lower ESR values than did alcohol abstainers and individuals with low physical activity, respectively.

ESR varies greatly with age and sex, and corresponding reference values are proposed. Lifestyle factors (physical activity, smoking, and alcohol consumption) and common metabolic abnormalities (obesity and related metabolic syndrome) may also influence ESR values.

Abbreviations: BMI = body mass index, ESR = erythrocyte sedimentation rate, GAMLSS = generalized additive models for location scale and shape, MCV = mean corpuscular volume, MET = metabolic equivalents of task.

Keywords: age, alcohol, erythrocyte sedimentation rate, gender, metabolic syndrome, obesity, physical exercise, smoking

Medicine (Baltimore). 2019 Aug; 98(34): e16816.
Published online 2019 Aug 23. doi: 10.1097/MD.0000000000016816

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Link:https://www.topstudyworld.com/2020/05/access-hec-digital-library.html?m=1

References

Sr.#	Types of reference material	Names/links of reference material
1	Textbook	Guyton AC, Hall JE. Textbook of Medical Physiology. 14th ed. Philadelphia, PA: Saunders; 2020
2	Textbook	Boron WF, Boulpaep EL. Medical Physiology: A Cellular and Molecular Approach. 3rd ed. Philadelphia, PA: Elsevier; 2017.
3	Textbook	Sherwood L. Human Physiology: From Cells to Systems. 10th ed. Boston, MA: Cengage Learning; 2020.
4	Textbook	Widmaier EP, Raff H, Strang KT. Vander's Human Physiology: The Mechanisms of Body Function. 16th ed. New York, NY: McGraw-Hill Education; 2021.
5	Textbook	Costanzo LS. Physiology. 7th ed. Philadelphia, PA: Saunders; 2021.
6	Research papers	https://doi.org/10.1097%2FMD.0000000000016816
7	YouTube Video	https://youtu.be/gy8RpErVsqc

Thank You