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ESR BASICS

Erythrocyte Sedimentation Rate

ERYTHROCYTE SEDIMENTATION RATE (ESR)

- ESR is one of the most performed laboratory tests in the world[1].
- ESR is a non-specific test that indirectly measures the presence of inflammation in the body.
- ESR reflects the tendency of red blood cells (RBCs) to settle more rapidly in some disease states usually because of increases of protein in the bloodstream.
  - In general, ESR is increased in acute, general infections and localized, acute, inflammatory conditions.
  - Changes in erythrocyte sedimentation can also be affected by RBC shape, number, and size[2].

ESR can be used as a…

- **Screening Test**
- **Test to aid in diagnosis**
- **Test to aid in differential diagnosis**
- **Test for monitoring a condition or response to therapy**

1. Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ; on behalf of the International Council for Standardization in Haematology (ICSH). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. Int J Lab Hem. 2017;39:448–457. https://doi.org/10.1111/ijlh.12693
2. CLSI. Procedures for the Erythrocyte Sedimentation Rate Test; Approved Standard— Fifth Edition. CLSI document H02-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

THE HISTORY OF ESR TESTING

- The ESR theory was first observed by Polish physician Dr. Edmund Faustyn Biernacki in 1897. Biernacki observed increased ESR values in patients that also had increased levels of fibrinogen, a plasma protein that promotes platelet aggregation.
- Following this discovery, in 1918, Swedish hematologist Dr. Robert Fahraeus studied the ESR values of pregnant and non-pregnant women. Fahraeus found that pregnancy caused an elevation in ESR (Aytekin, 2018).
- A few years after Fahraeus' discovery, in 1921, Swedish internist Dr. Alf Vilhelm Albertsson Westergren studied the ESR results of patients with pulmonary tuberculosis.
- Together, Fahraeus and Westergren developed the Fahraeus-Westergren method to measure ESR. This method – today referred to as the Westergren method – is regarded as the reference method in ESR testing by laboratories all over the world.

Dr. Robert Fahraeus

Dr. Alf Vilhelm Albertsson Westergren

ESR IN CLINICAL PRACTICE

ESR testing can be a **useful tool** that, alongside other testing & clinical findings, helps healthcare providers reach a diagnosis or evaluate a current condition state.

Screening

A sensitive indicator of inflammation, making it valuable for identifying underlying inflammatory conditions even in the absence of specific symptoms.

Assisting Diagnosis

Aids in diagnosing various inflammatory and infectious diseases, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), and certain infections like tuberculosis.

Differential Diagnosis

In conjunction with other clinical findings and laboratory tests, it helps differentiate between different disease conditions with similar symptoms (e.g. rheumatoid arthritis & osteoarthritis).

Monitoring Therapeutic Changes

Can be monitored over time to assess a patient's response to therapy. Changes in ESR values may indicate the effectiveness of treatment or disease progression, enabling timely adjustments in patient management.

- Brigden ML. Clinical utility of the erythrocyte sedimentation rate. Am Fam Physician. 1999 Oct 1;60(5):1443-50. PMID: 10524488.
- Tishkowski K, Gupta V. Erythrocyte Sedimentation Rate. [Updated 2023 Apr 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557485/

CLINICAL UTILITY OF ESR

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There are numerous reasons why a differential diagnosis may cause an elevated ESR level indicating the severity of the inflammation process. These may include:

Detect Inflammation

- Multiple Myeloma
- Polyclonal hypergammaglobulinemia
- Hyperfibrinogenaemia
- Lupus
- Rheumatoid arthritis

Diagnosis and Monitoring

- Temporal Arteritis
- Polymyalgia Rheumatica
- Side effects of drug therapy

Oncology

- May correlate with poor prognosis in many cancers
- ESR >100 mm/hr can indicate metastatic disease in solid tumor patients

**Note this is not an exhaustive list of conditions*

Clinical and Laboratory Standards Institute (2011), Procedures for the Erythrocyte Sedimentation Rate Test: Approved Standard – Fifth Ed. H02-A5, Vol. 31 No. 11 pp.12

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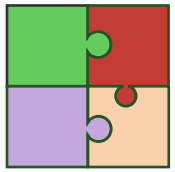
ESR AND CRP ARE COMPLEMENTARY, NOT INTERCHANGEABLE



Both are **non-specific** and should be combined with other tests and patient history[1,2]

ESR improves clinical decision-making when results are discordant with CRP[3]

ESR detects inflammatory disorders not detected by clinical examination or CRP[3]

ESR better reflects cumulative inflammation over days to weeks, useful for chronic or subacute conditions[4]

ESR is more sensitive to diffuse, low-grade/subacute conditions, indicating systemic inflammation[1]

ESR is less sensitive to short term fluctuations and more stable in chronic disease monitoring[1]

ESR has a unique kinetic profile complementary to CRP[1]

1. Litao MKS, Kamat D. Erythrocyte Sedimentation Rate and C-Reactive Protein: How Best to Use Them in Clinical Practice. *Pediatric Annals.*2014;43(10):417-420.
2. Markanday A. Acute Phase Reactants in Infections: Evidence-Based Review and a Guide for Clinicians. *Open Forum Infect Dis*.2015 Jul 3;2(3):ofv098.
3. Singh G. C-reactive protein and erythrocyte sedimentation rate: Continuing role for erythrocyte sedimentation rate. *Advances in Biological Chemistry.*2014;4:5-9.
4. Harrison M. Erythrocyte sedimentation rate and C-reactive protein. *Aust Prescr*. 2015 Jun;38(3):93-4.

ESR REPORTING RANGES & INFLUENCES

Mean ESR Reference Values for the Westergren Method (mm/hr)*			Upper Limit of Normal	
Age (Years)	**Male**	**Female**	**Male**	**Female**
18-30	3.1	5.1	< 7.1	< 10.7
31-40	3.4	5.6	< 7.8	< 11.0
41-50	4.6	6.2	< 10.6	< 13.2
51-60	5.6	9.4	< 12.2	< 18.6
60-70	5.6	9.4	< 12.7	< 20.2
Over 70	5.6	10.1	< 30	< 35

Ranges are provided for reference only. Each lab should establish a protocol for verifying ranges

Physiological and clinical factors that **DECREASE** the ESR

Conditions that cause delayed stacking of erythrocytes or abnormally shaped erythrocytes such as polycythaemia and sickle cell anaemia. Increases in plasma viscosity can also impede the speed of erythrocyte sedimentation.

Physiological and clinical factors that **INCREASE** the ESR

Conditions that cause increased levels of immunoglobulins, fibrinogen or other clotting factors.

Clinical and Laboratory Standards Institute (2011), Procedures for the Erythrocyte Sedimentation Rate Test: Approved Standard – Fifth Ed. H02-A5, Vol. 31 No. 11 pp.12

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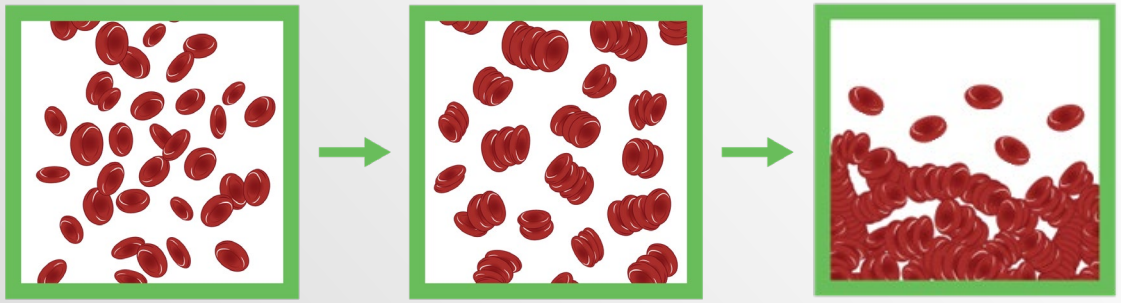
PHYSIOLOGY OF ESR

Erythrocyte Sedimentation Rate

ESR IS A PHYSIOLOGICAL REACTION

- Unlike other laboratory tests that are detecting an analyte with a specific molecular structure, ESR is measuring a "physiochemical phenonemon"[1]
- The ESR phenomenon is transient and confined to fresh blood.
- ESR procedures cannot be calibrated, and methods for measuring ESR are naturally susceptible to a variety of errors[2].
- There are a number of environmental and sample variables that can affect ESR since it is a measure of RBC behaviour and not a concentration.

Erythrocyte Sedimentation Process



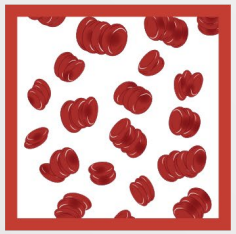
Red blood cell sedimentation is a multi-phase process, and different ESR methods measure different parts of this process

1. Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ; on behalf of the International Council for Standardization in Haematology (ICSH). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. Int J Lab Hem. 2017;39:448–457. https://doi.org/10.1111/ijlh.12693
2. CLSI. Procedures for the Erythrocyte Sedimentation Rate Test; Approved Standard— Fifth Edition. CLSI document H02-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

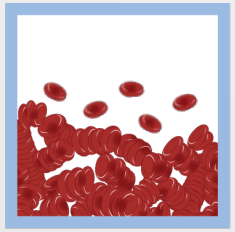
ERYTHROCYTE SEDIMENTATION PROCESS

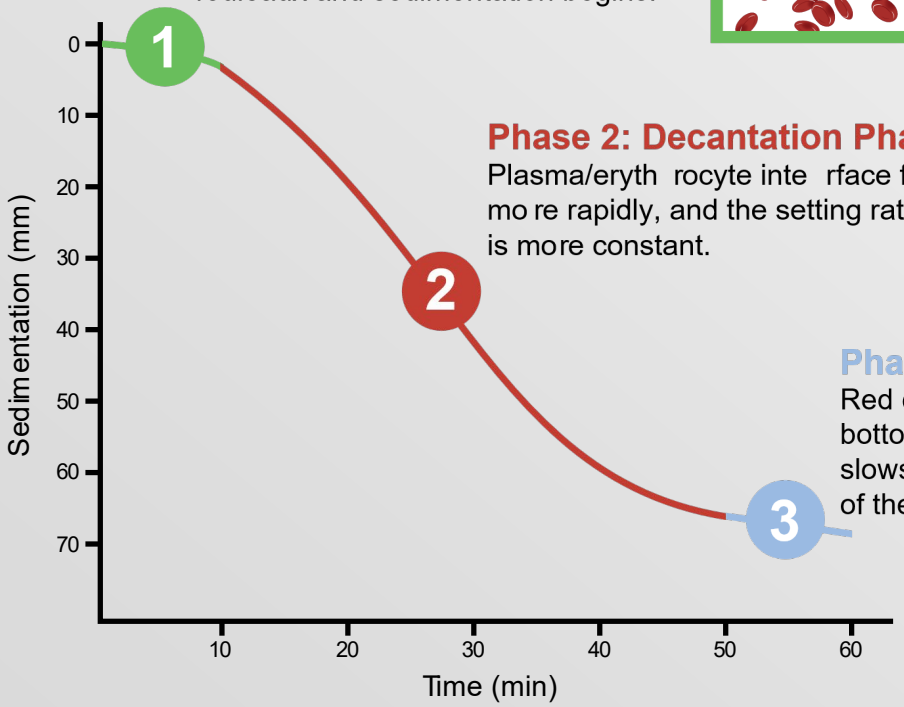
Phase 1: Lag Phase -
Erythorocytes aggregate to form rouleaux and sedimentation begins.

Phase 2: Decantation Phase -
Plasma/erythrocyte interface falls more rapidly, and the setting rate is more constant.



Phase 3: Packing Phase -
Red cell aggregates pile up at the bottom of the tube. The sedimentation slows down as a result of the interference of the accumulated RBCs.



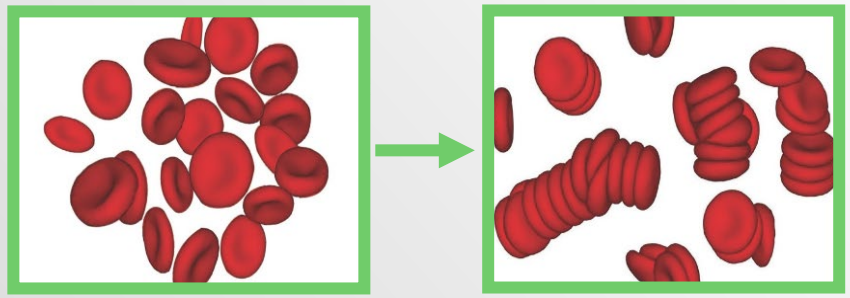


- Red blood cells do not actually fall at a true and constant rate
- The degree of aggregation is directly proportional to the degree of sedimentation because **sedimentation cannot occur without aggregation**

CLSI. Procedures for the Erythrocyte Sedimentation Rate Test; Approved Standard— Fifth Edition. CLSI document H02-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

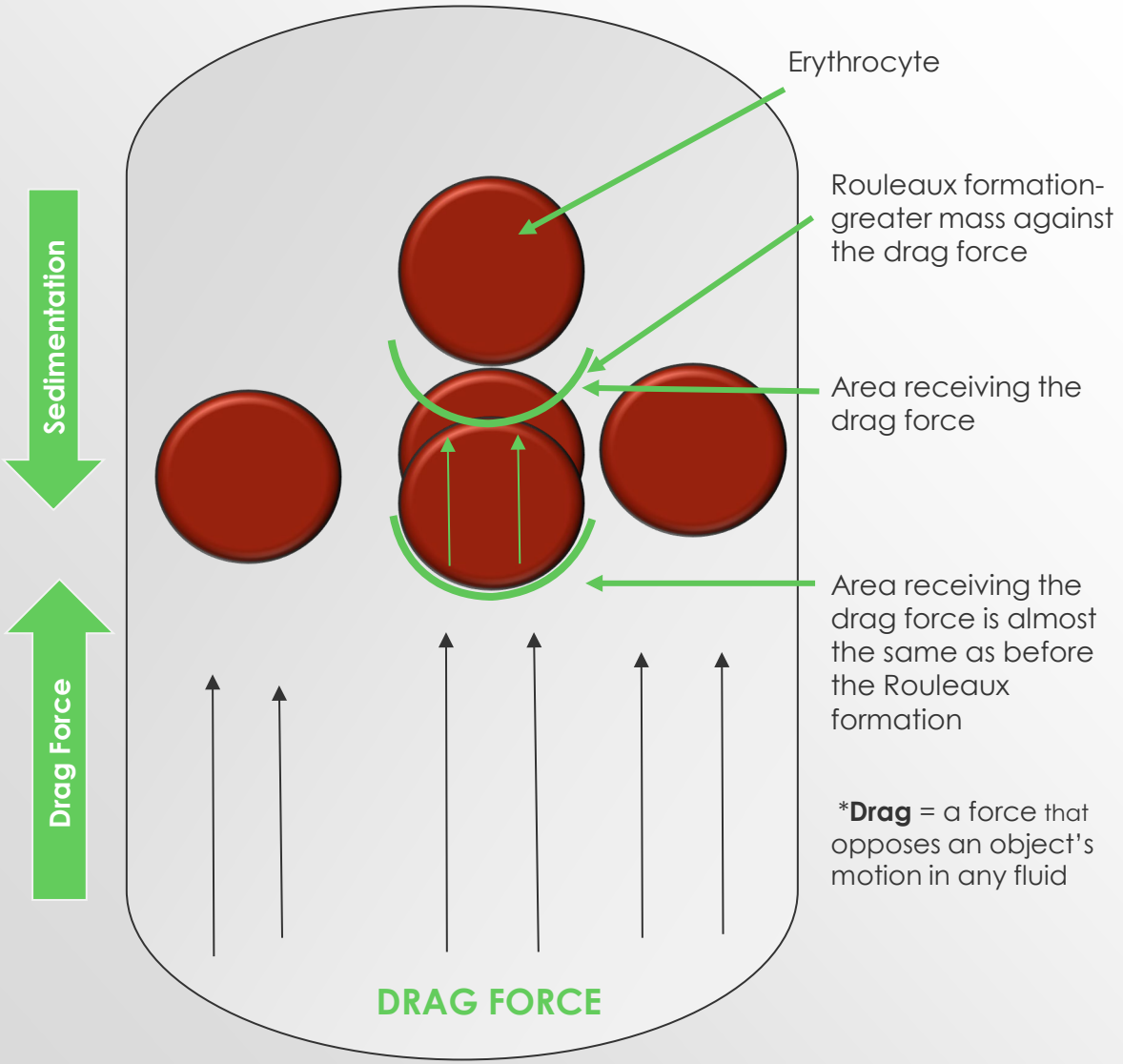
PHASE 1: LAG PHASE

- Measuring the ESR indirectly gauges the intensity of red cell aggregation.
- The positive charges of the proteins (from inflammatory processes) bind with negatively charged red blood cells. This causes red blood cells to aggregate and form ‘rouleaux’.
- Rouleaux are stacks of erythrocytes. Their formation is promoted by acute phase reactants and immunoglobulins and is hampered by albumin.



PHASE 2: SEDIMENTATION PHASE

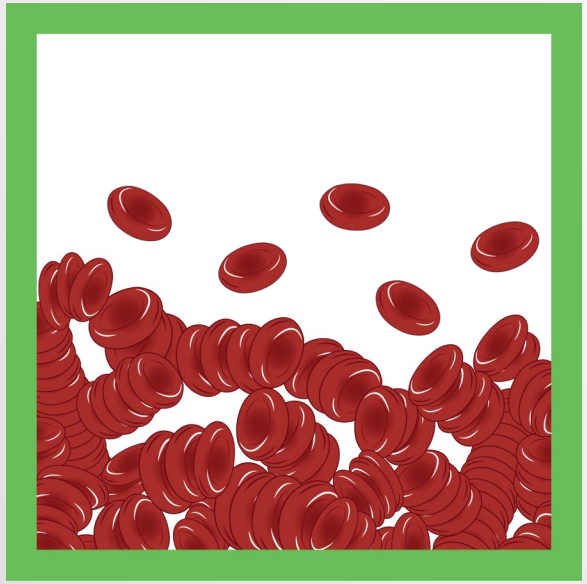
- If inflammation is present, acute phase reactants will bind to red blood cells, creating rouleaux, and the cells will sediment.
- With rouleaux formation, the weight of the stacked red blood cells is greater than the plasma surrounding the stacked cells, causing them to sediment.
- As red blood cells begin to sediment, a drag force is applied to the outside of the cell as it starts to fall or sediment.



1In 1851, George Gabriel Stokes derived an expression, now known as Stokes law, for the frictional force – also called drag force – exerted on spherical objects with very small Reynolds numbers in a viscous fluid. Stokes, G. G. (1851). "On the effect of internal friction of fluids on the motion of pendulums". Transactions of the Cambridge Philosophical Society. 9, part ii: 8–106.

PHASE 3: PACKING PHASE

- The faster the aggregation occurs, the larger the Rouleaux formations become, and the faster the red blood cells fall or sediment by gravity (Stokes' law).
- During the final packing phase, sedimentation slows down and the red blood cells begin to pack at the bottom.
- Rouleaux sediment faster than erythrocytes. The degree of rouleaux formation is directly proportional to the sedimentation rate and one of the major determinants of ESR.



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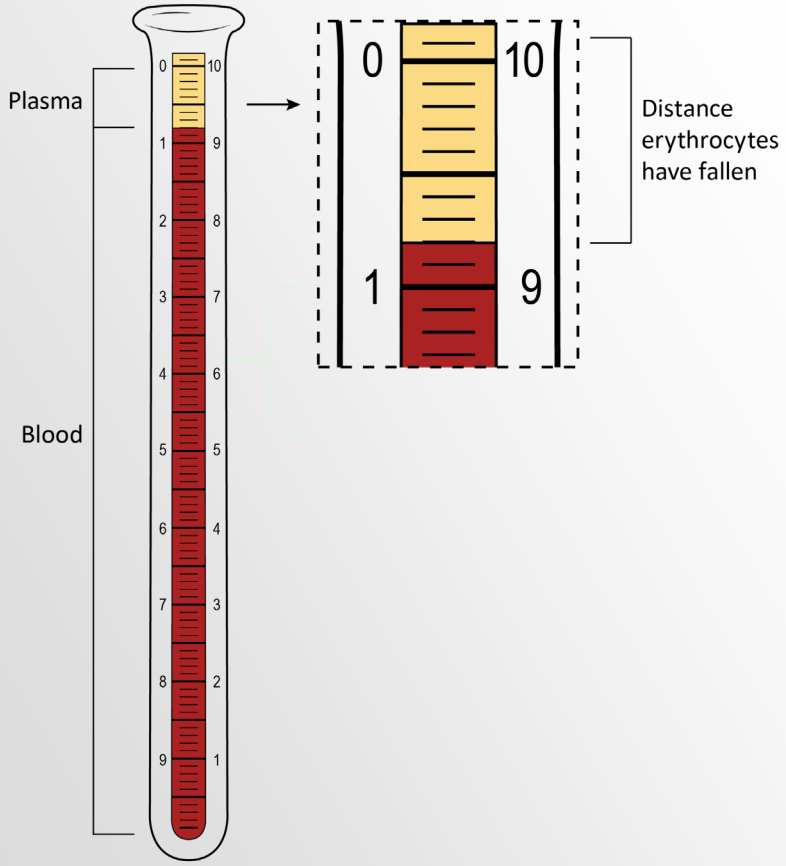
METHODOLOGY

The science behind ALCOR Scientific's ESR analyzers

THE WESTERGREN METHOD

- Traditionally, ESR has been performed using the Westergren method which is still endorsed as the reference method by CLSI and ICSH.
- The manual Westergren method of performing ESR has remained essentially unchanged since its inception.
  - Anticoagulated blood is placed in a specialized graduated vertical tube and left to sit for 60 minutes.
  - The Westergren method is reported out in mm/hr; traditionally this has been the distance measured from the bottom of the meniscus to the top of the RBC sediment.
- There are different products on the market for performing the Westergren method; multiple guidelines have attempted to define a standardized procedure to help reduce variability in ESR results.



- Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ; on behalf of the International Council for Standardization in Haematology (ICSH). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. Int J Lab Hem. 2017;39:448–457. https://doi.org/10.1111/ijlh.12693
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ICSH WESTERGREN PROCEDURE

Blood Collection

Non-hemolyzed blood is anti-coagulated with EDTA at collection. It is recommended that the **EDTA sample is tested within 4 hours after** collection, but it has been reported that **storage for up to 24 hours** at 4°C still results in a stable ESR value. When ready to test, the blood sample is thoroughly **mixed and diluted 4:1** using a sodium citrate solution.

Tube Handling

Uses standardized colourless, circular glass or plastic tubes with sufficient length to include 200 mm sedimentation scale. **The diluted sample is aspirated and transferred to the Westergren tube.** The Westergren tube is then placed in a stable, vertical position at a **constant temperature between 18°C and 25°C in an area free from vibrations, drafts and direct sunlight.**

Reading the Result

After 60 minutes, the distance from the bottom of the plasma meniscus to the top of the descended erythrocytes(red blood cells) is read **and recorded in mm per hour.** The buffy coat that is made up of leukocytes should not be included in the erythrocyte column.

Jou JM, Lewis SM, Briggs C, Lee SH, De La Salle B, McFadden S; International Council for Standardization in Haematology. ICSH review of the measurement of the erythocyte sedimentation rate. Int J Lab Hematol. 2011 Apr;33(2):125-32. doi: 10.1111/j.1751-553X.2011.01302.x. Epub 2011 Feb 25. PMID: 21352508.

LIMITATIONS OF THE WESTERGREN METHOD

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Environmental variables

The Westergren method is subject to several environmental variables while the red cells sediment over 60 minutes, such as room temperature, humidity, and benchtop vibrations

Workflow

The Westergren method is a labour-intensive manual procedure

Restrictive sample requirements

Samples must be tested or refrigerated within 4 hours which can prove difficult when sample collective sites are dispersed

Operator error and subjectivity

Due to the manual nature of the test, there are many opportunities for human error including sample mixing, tube labelling, sample dilution, tube setup, result recording, etc., and technique varies between operators

Biohazard risk

The Westergren method requires users to handle a blood sample, introducing the risk of exposure to blood-borne pathogens

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A GLOBAL NEED FOR LABORATORY EFFICIENCY

- While the Westergren method remains the reference method for ESR, a variety of new ESR methods have been introduced to the market since 2001.
- The Modified Westergren and Alternate ESR methods attempt to overcome the serious limitations of the Westergren method.

When global EQA data was review by the ICSH working group in 2017, 72% labs were using modified Westergren or alternate ESR methods

ESR methods can be classified into 3 categories

- **Westergren methods**
- **Modified Westergren methods**
- **Alternate ESR methods**

Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ; on behalf of the International Council for Standardization in Haematology (ICSH). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. Int J Lab Hem. 2017;39:448–457. https://doi.org/10.1111/ijlh.12693

THE EVOLUTION OF ESR TESTING

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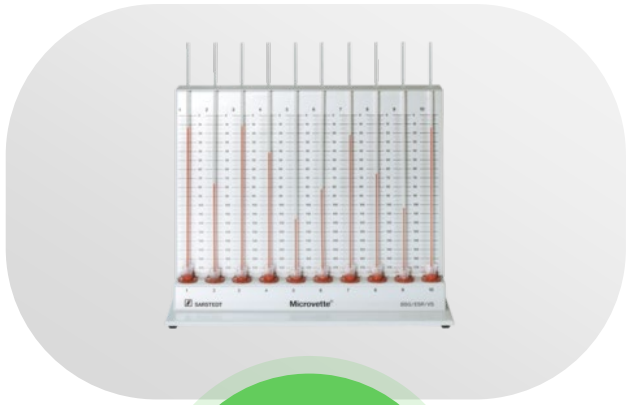

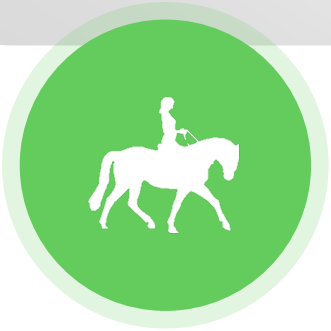

Westergren Method

First discovered in 1827 and used in clinical practice since 1918. Requires exposure to blood, labor intensive and the result is susceptible to many variables. Requires 60 minutes for blood to settle.

Semi-Automated

Based on Westergren method with various modifications such as diluents, secondary tubes, centrifugation. Shorter time to result than Westergren (varies by method).

The Next Generation

Fully automated, standardized, and safer STAT ESR testing with realistic sample stability.

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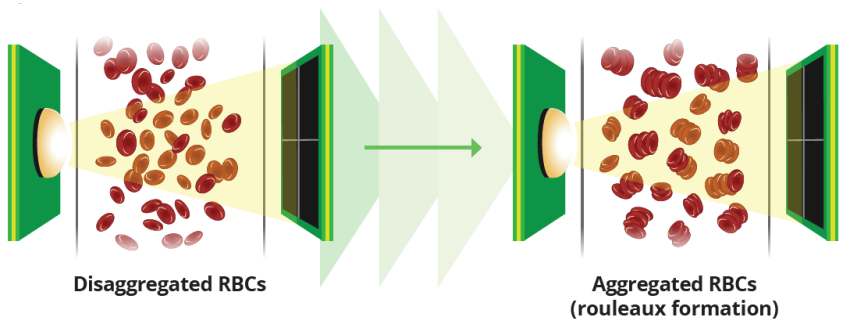
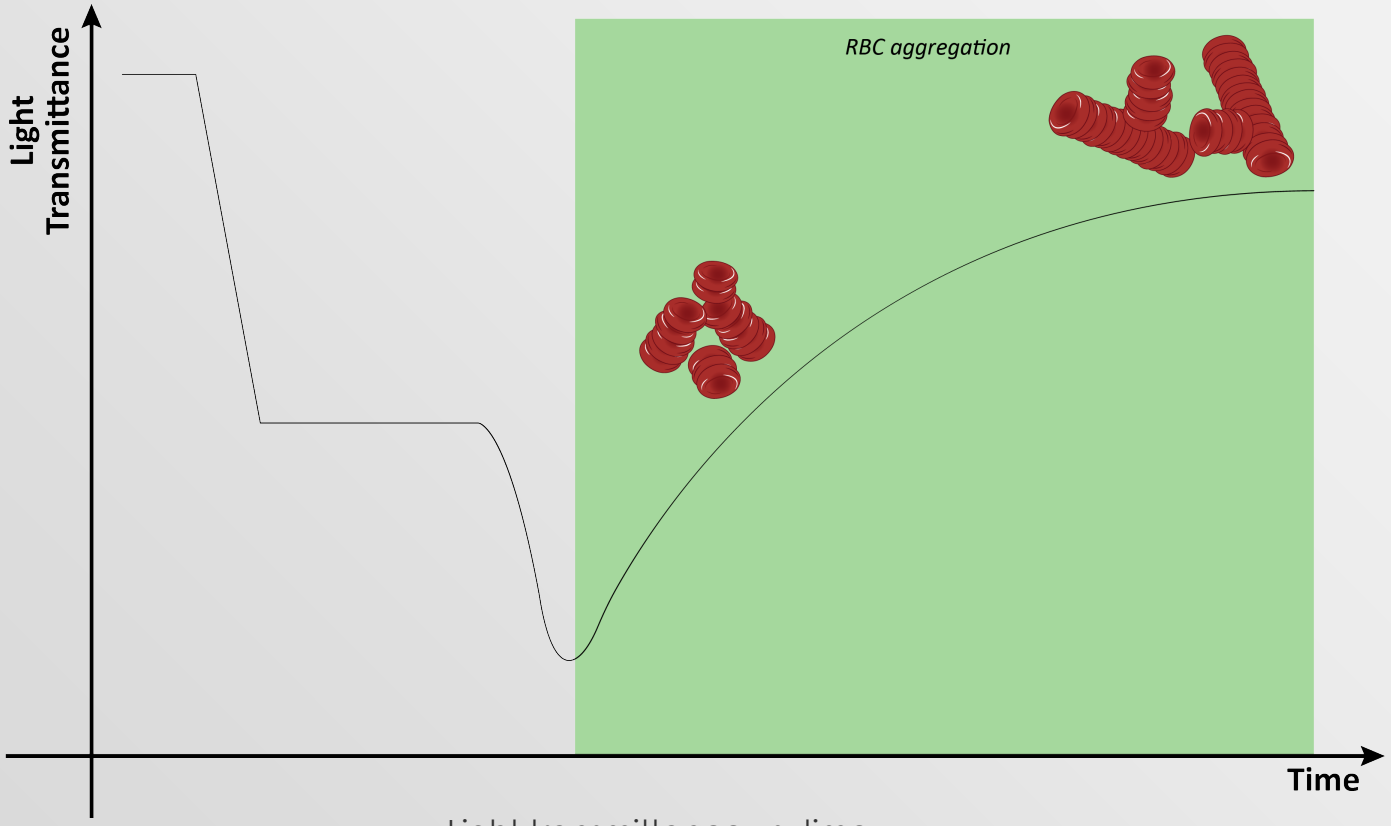
iSED® TECHNOLOGY

- The iSED analyzers use to measure ESR by employing syllectometry to assess RBC behaviour.
- A blood sample is pumped into the flow cell (which disaggregates the RBCs), and light transmission is measured as RBCs begin to reaggregate and form rouleaux.
- More aggregation results in more light transmission through the flow cell.
- RBC aggregation begins within seconds, so iSED analyzers can provide an ESR result within 20 seconds (after appropriate sample mixing).



Giarrusso S, Langella J, Frappa F, McCutcheon M. iSED ELITE: Using rheology to expedite ESR testing. Poster presented at ISLH meeting; May 11, 2023; New Orleans, LA.



Light transmittance vs. time

UNDERLYING SYLLECTOMETRY

- In syllectometry, light transmission is plotted over time to generate a syllectogram.
- The iSED family of analyzers generate and mathematically analyze a syllectogram to determine the degree of RBC aggregation.
  - Light transmission increases when RBCs form rouleaux because RBC surface area decreases.
  - The reading algorithm is based on the speed and magnitude of the aggregation kinetic of fresh blood samples.
  - Results are converted to mm/hr.

Giarrusso S, Langella J, Frappa F, McCutcheon M. iSED ELITE: Using rheology to expedite ESR testing. Poster presented at ISLH meeting; May 11, 2023; New Orleans, LA.

iSED TECHNOLOGY IMPROVES SAMPLE STABILITY

The extended ESR sample stability afforded by the iSED family of analyzers is more attainable for today's busy labs.

- Samples tested with the iSED photometric rheology technology are significantly more stable than Westergren-based ESR methods.
- iSED analyzers are measuring aggregation, not sedimentation, which is stable for longer.
- ESR samples tested on the iSED family of analyzers are stable up to **28 hours at room temperature (18-25°C) and 48 hours refrigerated (2-4°C).**

Traditional Westergren sample stability:

4 hours at room temperature

24 hours refrigerated

ESR TRAINING TAKEAWAYS

- Erythrocyte sedimentation is a physiochemical phenomenon.
- ESR is used for assessing inflammation; it is used in conjunction with other testing and clinical factors for diagnosis or monitoring.
- The Westergren method is the reference method for ESR according to the guidelines, however there is a global need for improved workflows and reduction of environmental variability, reporting variability, and biohazard risk.
- The iSED family of products are considered an "alternate ESR method" as they use photometric rheology to measure red blood cell aggregation providing results in 20 seconds.

Jou JM, Lewis SM, Briggs C, Lee SH, De La Salle B, McFadden S; International Council for Standardization in Haematology. ICSH review of the measurement of the erythocyte sedimentation rate. Int J Lab Hematol. 2011 Apr;33(2):125-32. doi: 10.1111/j.1751-553X.2011.01302.x. Epub 2011 Feb 25. PMID: 21352508.

THANK YOU!

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