A classic, gold standard:

The Westergren method for ESR measurement

RR Mechatronics
Masters of Measurement

WP-001, rev. 005

Executive Summary

Erythrocyte Sedimentation Rate (ESR) is a sensitive, non-specific marker of inflammation. ESR is used as a "general physical condition" marker, in combination with clinical history, physical examination and other standard laboratory tests. It can serve as a guide to aid diagnosis, management and follow-up of different autoimmune diseases, acute and chronic infections and tumors.

Modern and fully automated instruments, like the Starrsed, have made the ESR test even more accurate and safe in comparison with the manual Westergren version. Several published studies highlight that alternatives to the ICSH and CLSI declared gold standard ESR method, that use a test principle that is very different from Westergren, give rise to a large percentage of false negatives and thus a risk of missed diagnoses.

This white paper provides an overview on the history and measurement of the ESR and the different tests/instruments available on the market. In this paper shows RR Mechatronics features its Starrsed Line of fully automated Westergren ESR instruments. It shows several publications on analysis of these tests in comparison with the gold standard of Westergren (and Starrsed). It sheds light on the importance of the automated, accurate and safe use of the Starrsed.

White Paper by RR Mechatronics

Published February 2018

Key Findings

- The erythrocyte sedimentation rate (ESR) is a non-specific marker, and is, in combination with clinical history and physical examination used as a general condition indicator. It is a classic test that measures how far erythrocytes settle in a test tube over the course of time; 60 ± 1 minute. *(page 4, 15)*
- Erythrocyte sedimentation is governed by factors that stimulate or inhibit erythrocyte aggregation and sedimentation. The clinically most relevant factors that influence ESR are the erythrocytes themselves and plasma proteins associated with inflammation and tissue damage. Erythrocytes usually aggregate into clumps that resemble a stack of coins and are called rouleaux. *(page 4,5)*
- The Westergren method as described by the Clinical and Lab Standards Institute (CLSI) is the gold standard and reconfirmed in 2017 as the reference method for ESR measurement by the International Council for Standardization in Hæmatology (ICSH). *(page 6,7)*
- In the original Westergren method, the ESR is read after 60 minutes. An ESR reading after 30 minutes can reliably be extrapolated to the corresponding ESR reading at 60 minutes (correlation coefficient = 0.984). The Starrsed ESR can work in 30 and 60 minutes mode *(page 10)*
- Test-1 and iSED are ESR analyzers that produce ESR reading results within 20 seconds after sampling. It takes however approximately 10 minutes before sedimentation starts at a constant rate. This means that the Test-1 analyzer doesn't actually measure sedimentation, but rather calculates a mathematically derived ESR, based on aggregate measurements in the first, rouleaux forming stage only. *(page 11,12)*
- The Test-1, the iSED and the VES-Matic demonstrate clear flaws compared to the original Westergren and the Starrsed, leading to an important number of false negatives. *(page 12)*
- ICSH recommends to: Consider adding an interpretative comment to every result stating that "This result was obtained with an ESR instrument that is not based on the standard Westergren method. The sensitivity and specificity of this method for various disease states may be different from the standard Westergren method". *(page 12)*
- The Starrsed ESR analyzers from RR Mechatronics are automated ESR analyzers that use the reference Westergren method as recommended by the ICSH and CLSI. *(page 11)*
- The Starrsed automated implementation of the Westergren ESR also takes care of the many things that might influence the quality of the test result, for example: temperature, stability, dilution, washing and drying of the Westergren tubes and detecting problematic (hemolytic) samples. *(page 6,7,8,12)*
- ESR measurement is useful in the diagnosis of rheumatoid arthritis, temporal arthritis, polymyalgia rheumatica, multiple myeloma and several autoimmune diseases. Clinical studies have also suggested possible relevance of ESR levels in different other conditions. *(page 15,16)*

Erythrocyte Sedimentation Rate

The erythrocyte sedimentation rate (ESR) is a non-specific marker, used as a general condition indicator. ESR is a non-specific marker of inflammation. It can be used in combination with the patient's clinical history and physical examination and can serve as a guide to aid diagnosis, management and follow-up of different auto-immune diseases, acute and chronic infections and tumors (Bridgen, 1999).

ESR is a classic test that measures how far erythrocytes settle in a test tube over the course of time. For this test, anti-coagulated whole blood is allowed to settle in an upright tube under standardized conditions. The ESR is the distance in mm that the erythrocytes have fallen during that time. There are many factors that affect the ESR, but the most clinically relevant factors that influence ESR are the erythrocytes themselves and plasma proteins associated with inflammation and tissue damage.

The discovery of ESR

Although Alf Westergren often is associated with ESR, he was not the first to notice the significance of ESR or to attempt to develop a method for measuring ESR.

The first one to notice and record changes in blood sedimentation during inflammation was John Hunter, who mentioned this in "A treatise on the blood, inflammation and gunshot wounds", that was published posthumously in 1794. About a century later, in 1897, Edmund Biernacki, a Polish physician, noticed that ESR was influenced by fibrinogen and developed his own ESR test, which he published and presented to his peers. But since he published his findings in Polish and German journals, his observations were hardly noticed in the English-speaking world.

In the 1920's, Swedish pathologist Robert Fåhræus and physician Alf Westergren made similar observations of the ESR in pregnant and tuberculosis patients. Together, they developed the Fåhræus-Westergren method of measuring ESR, which was quickly and widely adopted in clinical laboratories over the world and became known as the Westergren method.

(Madrenas J, 2005) (Grybowski & Sak, 2011 ;38)

Erythrocyte sedimentation process

Erythrocyte sedimentation is governed by factors that stimulate or inhibit erythrocyte aggregation and sedimentation. Normal erythrocytes are negatively charged and repel each other, which limits the sedimentation rate. Large clumps fall faster than small ones, so factors that increase aggregation will increase sedimentation. Erythrocytes usually aggregate into clumps that resemble a stack of coins, which are called rouleaux.

Normal Erythrocytes Inflammation

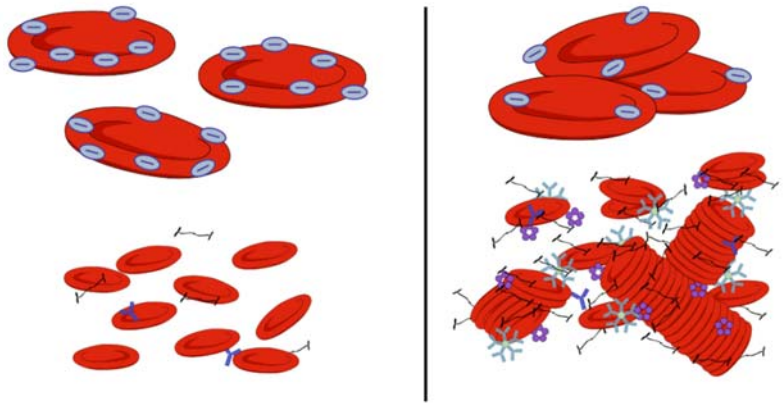


Fig 1: Normal: Negatively charged erythrocytes; low sedimentation rate.
Inflammation: Less negatively charged erythrocytes; sedimentation occurs, stimulated by all the different factors that increase rouleaux formation (Fibrinogen, CRP, Immunoglobulin).

The sedimentation process can be divided into three stages:

A. Lag stage-rouleaux formation (0-20 min) Erythrocytes start to aggregate and form rouleaux. The presence of acute phase proteins encourages rouleaux formation. During this phase, no sedimentation occurs.

B. Decantation stage-sedimentation (15-30 min) Erythrocyte aggregates fall to the bottom under influence of gravity at a constant rate. Large aggregates fall faster than small aggregates or single cells. Falling aggregates induce an upward plasma current that slows down sedimentation.

C. Packing stage (25-60 min) The rate of sedimentation slows down to zero and cells start to pack in the bottom of the tube.

Rouleaux formation

Fig. 2: At low or no flow condition, RBC's adhere side to side and form stacks called rouleaux, followed by end to end connections creating 3D aggregates (the rouleaux formation)

(Fabry, 1987)

The Westergren method is the gold standard

In the 1920's, Swedish practitioners Robert Fårhæus and Alf Westergren developed a systematic method for ESR measurement. Although several alternative methods were developed in that era, the Fårhæus-Westergren method, or Westergren method as it became known in the English-speaking world, quickly gained dominance. In 1973, the Westergren method was adopted as the reference method for ESR measurement by the International Council for Standardization in Hæmatology (ICSH). The Westergren gold standard was reconfirmed in 2017 (Kratz et al., 2017) by the ICSH and by the Clinical and Laboratory Standards Institute (CLSI). It remains the gold standard that all other ESR measurement methods and techniques are evaluated against.

The Wintrobe method: An alternative method for ESR measurement

The sixth edition of Gradwohl's Clinical Laboratory Methods and Diagnosis, published in 1963, mentions five different methods to measure ESR. These were the Westergren method, the Linzenmeier method, the Graphic or Cutler method, the Wintrobe-Landsberg method and the Landau method, which was a modification of the Linzenmeier method.

Of these methods, only the Westergren method and Wintrobe method are still in use today. The Wintrobe method uses tubes of only 100 mm long with a smaller diameter than standard Westergren tubes. EDTA blood without extra diluent is added to the tube and allowed to sediment for 60 minutes. After 60 minutes the distance that the blood cells have fallen is registered in mm.

Because the Wintrobe tubes are shorter than the Westergren tubes, the method is less sensitive than the Westergren method.

(Frankel, Reitman, & Sonnenwirth, 1963)

Procedure

The Westergren method as referenced by the ICSH consists of the following steps:

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

The Westergren method uses standardized colorless, circular glass or plastic tubes, with an inner diameter of at least 2.55 mm and sufficient length to include a 200 mm sedimentation scale. The inner diameter should be constant (± 5%) over the whole length; a so called Westergren tube.

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 (± 1°C) between 18°C and 25°C in an area free from vibrations, drafts and direct sunlight.

Reading the result

After 60 ± 1 minute, the distance from the bottom of the plasma meniscus to the top of the descended erythrocytes is read and recorded in mm. The buffy coat that is made up of leukocytes should not be included in the erythrocyte column.

(Jou, Lewis, Briggs, Lee, De La Salle, & McFadden, 2011) (CLSI, 2011)

The Westergren method

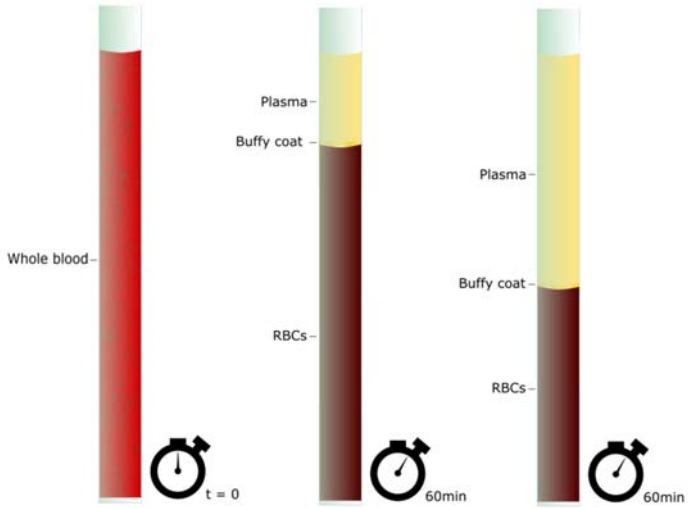


Fig 3: a. The diluted sample is aspirated and transferred to the Westergren tube.
b. A normal ESR after 60 minutes; <20 mm plasma.
c. An elevated ESR after 60 minutes.

Technical issues affecting ESR

Although the procedure is a simple one in theory, there are several technical factors that can affect the ESR result.

Quality of the Westergren tubes

Tubes with inadequate bore holes can be sensitive to erratic plugs of tightly packed cells that cause undue variation in the ESR results, especially in blood samples of high ESR and with a high hematocrit volume.

Because of contamination risks, disposable tubes are sometimes used. These need to be replaced after use and generate a lot of waste. Some plastic tubes can have a strong attractive force on erythrocytes or release plasticizers that can affect sedimentation rate. Injection-molding release-agents used in the manufacturing process may sometimes alter sedimentation characteristics. Reusable tubes need to be cleaned thoroughly after use.

Sample storage

The storage conditions of the sample are permitted, conform the CLSI standard (CLSI, 2011), to be up to 4 hours at room temperature or up to 24 hours at 4 degrees Celsius in cold storage. These storage conditions are in line with the sample storage requirement for the hematology analyzers.

Incorrect blood preparation

Correct blood preparation is important for reliable results. Whole blood should be anti-coagulated with EDTA without significant dilution of the sample. Alternatively, blood can be collected and diluted 4:1 in special sodium citrate tubes suitable for ESR measurement. Heparin can lead to falsely increased ESR readings (Penchas, Stern, & Bar-Or, 1978).

Changes in plasma viscosity and hematocrit can cause variable plugging of the long Westergren tube by rapidly falling erythrocyte aggregates. Correct dilution (4:1) of the blood sample in sodium citrate prevents this and makes the measurement independent of viscosity and hematocrit differences.

Deviation in vertical placement

Westergren tubes need to be placed perfectly vertically. Angles of more than 2 degrees off the vertical can accelerate sedimentation and result in a false increase in ESR. A deviation of 3 degrees can accelerate ESR up to 30%.

Temperature variation

The sedimentation process is substantially influenced by temperature variations. One example would be when sunlight would shine on some tubes but not others. The ICSH recommends a constant temperature (± 1°C) between 18°C and 25°C.

Vibrations

Vibrations can artificially increase sedimentation rate and should therefore be avoided.

Problematic samples

In some cases, erythrocyte abnormalities can result in hazy, cloudy samples that are difficult to read. Also, hemolytic or lipemic samples can cause difficulties in the accurate reading of ESR. (CLSI, 2011) (Hardeman, Levitus, Pelliccia, & Bouman, 2010)

In some cases, **sampling** errors, e.g. a low sample volume, can lead to foam or bubbles in the sample. If the analyzing system does not perform internal quality controls and flags low quality samples and unreliable readings, the reduced quality of the sample can lead to incorrect results and false clinical interpretation.

Other possible sedimentation results

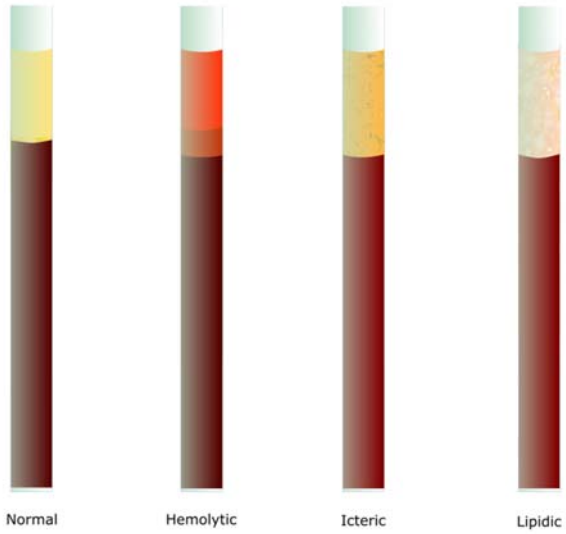


Fig.4: *- Normal sample*
- Hemolytic sample: Due to hemolysis; hemoglobin leaks from the damaged erythrocytes and turns the plasma red.
- Icteric sample: Mostly due to liver problems, elevated bilirubin turns the plasma darker yellow.
- Lipidic sample: Because of too much fat, the plasma turns opaque white and thickens.

Using tilt to speed up ESR

A tube that is not held completely vertical can lead to increased sedimentation rates and is one of the technical factors that can affect ESR readings. But could this knowledge be used to increase ESR and develop a rapid ESR method?

DM Dissanayake of the University of Peradenya in Sri Lanka has tested whether it was possible to use an inclined tube to get a faster reading of the ESR.

Dissanayake tilted tubes at an angle of 45 degrees and registered sedimentation distances every 30 seconds from 4 to 13 minutes by reading the lowest level of the meniscus. These results were compared with a traditional Westergren reading of the same sample in another tube that was kept vertically. The experiment contained a wide range of ESR readings, from 0 mm to well over 150 mm. The correlation between the traditional Westergren reading and the tilted tube was maximal between 10 and 11.5 minutes (correlation coefficient=0.985-0.986) for both low and high ESR readings.

(Dissanayake, 2006)

The accuracy of the results was considered acceptable. It demonstrates however that a tilted tube has a strong influence on the optimal testing time.

Reducing analysis time

In the original Westergren method, the ESR is read after 60 minutes, which puts practical limitations on the workflow in clinical laboratories. A laboratory investigation comparing the Westergren ESR method readings of a wide range of blood samples at 30 minutes and 60 minutes showed that 30 minute ESR readings correlate highly with the corresponding 60 minute ESR readings over a wide range of blood samples (correlation coefficient = 0.984). Thus, an ESR reading after 30 minutes can reliably be extrapolated to the corresponding ESR reading at 60 minutes. (Rogers, 1994)

Correlation coefficient:

- The value of a correlation coefficient ranges between -1 and 1.
- The strongest linear relationship is indicated by a correlation coefficient of -1 or 1.
- The weakest linear relationship is indicated by a correlation coefficient equal to 0.

NB: The correlation coefficient 0.984 is considered a very strong linear relationship. The accuracy of the results was considered acceptable. It demonstrates however that a tilted tube has a strong influence on the optimal testing time.

Comparison between 30 and 60 minutes Westergren method

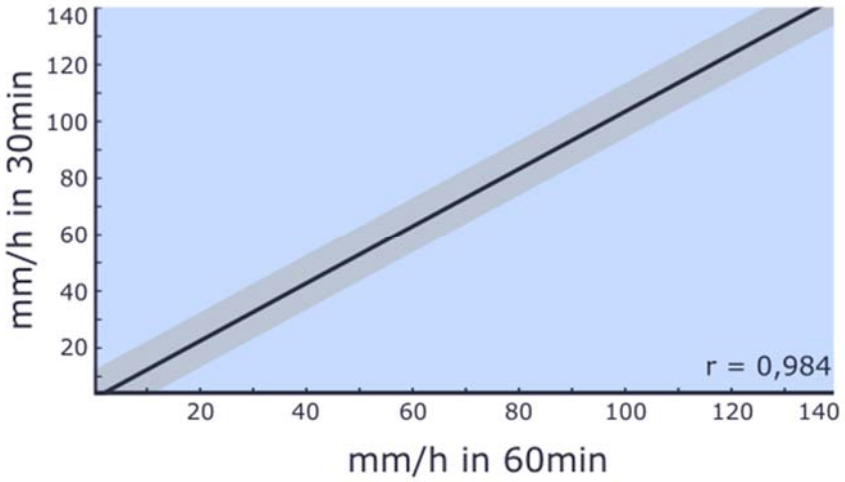


Fig. 5: The 30 minute ESR readings correlate highly with the corresponding 60 minute ESR readings over a wide range of blood samples (correlation coefficient = 0.984).

Aggregation versus sedimentation

Alifax Test-1 and Alcor iSED are ESR analyzers that produce ESR reading results within 20 seconds after sampling. Erythrocyte sedimentation is influenced by aggregation properties as well as plasma viscosity and hematocrit volume. It takes approximately 10 minutes before sedimentation starts at a constant rate. This means that the Test-1 and the iSED analyzers don't actually measure sedimentation, but rather calculates a mathematically derived ESR based on aggregate measurements in the first, rouleaux forming stage only. Thus, these test results need to be manipulated to an ESR value according to the Westergren method in order to be clinically useful.

The Diesse VES-Matic Cube line of instruments is like Westergren a sedimentation-based test. To run VES-Matic test it uses the original EDTA tube that was used for drawing the blood from the patient. No sample is taken from the tube and nothing is added. Not consuming any sample seems very attractive but has some serious drawbacks.

1. In order not to lose accuracy a relatively full sample tube is required. This puts some constraints on the testing order and logistics in the lab. Also the tube is occupied for at least 20 minutes before any other hematology test can be done.

2. A more serious drawback is that the sample in the primary EDTA tube is not diluted, nor is the result adjusted for the viscosity of the sample. The hematocrit level will have a significant influence on the measured sedimentation.

The hematocrit level of a sample will, among others, even vary with the individuals hydration level. Not adjusting for a variation in hematocrit (as is prescribed in Westergren) will make it impossible to truly compare the readings from the VES-Matic instruments with the ESR measures that are in accordance with the international standard as referenced by the ICSH.

Comparison of Westergren with other methods

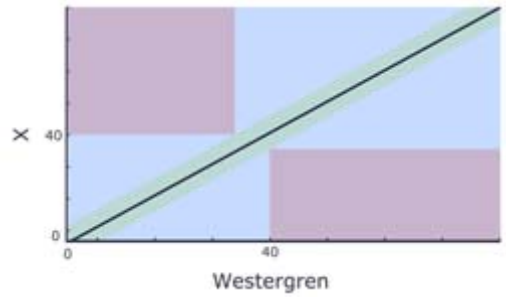
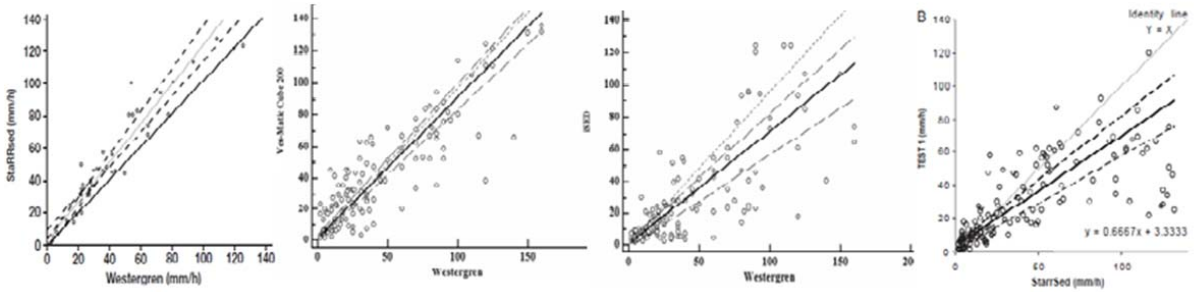


Fig. 6: Results reported into the upper left quadrant are considered normal according to the Westergren gold standard, but high according to method X. These so called "false positives" will lead to additional costs for supplementary testing or unnecessary treatment. Results reported into the lower right quadrant are considered high according to the Westergren gold standard, but low according to method X. These so called "false negatives" may lead to missed diagnosis.

In addition, sedimentation characteristics of the second and third stage can be relevant for some diseases, e.g. multiple myeloma. Test-1 was not as sensitive to the presence of paraproteins as the Westergren method (Raijmakers, Kuijper, Bakkeren, & Vader, 2008) and could produce significantly different results, especially in the higher ESR readings (Hardeman, Levitus, Pelliccia, & Bouman, 2010). In one comparison it was found that in 11.5% of the samples, the differences in results could lead to either a missed diagnosis (false negative) or additional testing costs (false positive) (Hardeman, Levitus, Pelliccia, & Bouman, 2010). Also, in diagnosing a flare in rheumatoid arthritis, Test-1 has shown to induce DAS28 misclassification in clinical practice (Maas et al., 2010).

The figures below are Passing Bablok regression plots taken from three independent publications. By evaluating and connecting the dots of three publications it is possible to compare the Test1 and iSED with the Starrsed and the gold standard Westergren. It again articulates the quality of the original Westergren method and the Westergren related methods in determining ESR.

The Test1, the iSED and the VES-Matic demonstrated clear flaws compared to the original Westergren and the Starrsed, leading to an important number of false negatives.



Regression Plots: comparing methods

Fig. 7: Higher than 40 on the Westergren scale and lower than 40 on the compared instrument scale are potentially missed diagnoses.

(Hardeman, Levitus, Pelliccia, & Bouman, 2010) (Raijmakers, Kuijper, Bakkeren, & Vader, 2008) (Bogdaycioglu, Yilmaz, Sezer, & Oguz, 2014) (Curvers, et al., 2010)

Recent ESR instrument evaluation articles

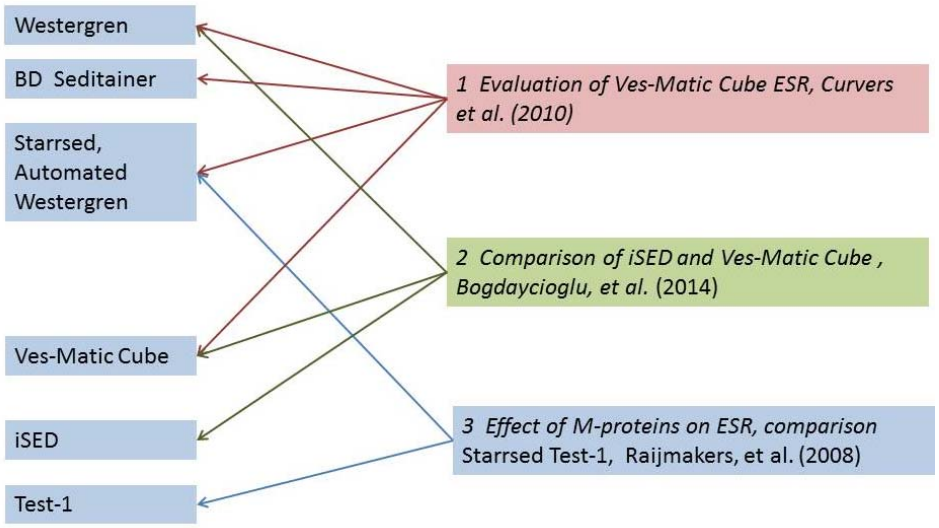


Fig. 8: Different publications evaluating different methods.

These observations point, according to the ICSH working group, toward consequences of the inherent differences between the Westergren method and the modified and alternate methods and the need for standardization and harmonization. The ICSH further points out that a laboratory using an alternate Westergren method should consider “adding an interpretive comment to every result that summarizes the sensitivity and specificity of the method for various disease states” (Kratz et al., 2017). In other words when using an “alternate Westergren” method, such as Test-1 or iSED, a laboratory should state that according to peer reviewed published research the results are not useful in the diagnosis of multiple myeloma or rheumatoid arthritis.

Quality equipment ensures reliable results and reduced cost of operation

The Starrsed ESR analyzers from RR Mechatronics are automated ESR analyzers that use the reference Westergren method as recommended in 2017 by the ICSH (Kratz et al., 2017) and CLSI (2011). Starrsed analyzers perform fully automated ESR measurements in 30 or 60 minutes.

Pre-mixing, sampling and dilution of standard whole-blood EDTA samples in sodium citrate is fully automated, which ensures accuracy and frees up time for the operator, who only needs to load the samples into the analyzer. The analyzers contain a built-in barcode reader that automatically identifies and registers the correct blood samples. Starrsed analyzers use a specifically designed needle for sampling that minimizes damage to the rubber stopper and ensures that blood vials can be sampled reliably multiple times.

Correct placement of a Starrsed analyzer guarantees a vertical position, a vibration-free environment and shielding from sunlight and drafts. The Starrsed analyzers use infrared light to read the ESR results and the optical reader is, in combination with built-in algorithms, even capable of detecting the relevant plasma-blood cell interface in hazy samples. The results are temperature corrected to 18.3°C and enable reliable clinical interpretation of the result.

Starrsed analyzers use standardized, reusable glass tubes that are specially made and tested. The tubes are cleaned using detergent and protease enzymes, rinsed and dried after each cycle, ensuring that the tubes are clean before use. This reduces waste and minimizes biohazard risks and the cost of operation.

<u>The washing process of the Westergren tubes</u>

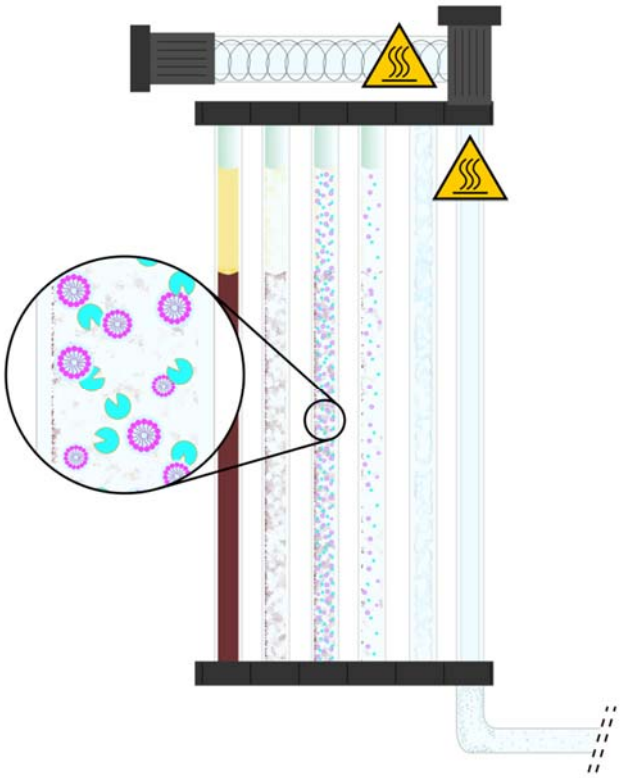


Fig. 9: The tubes are cleaned using detergent and protease enzymes. The inside of the tube is dried and disinfected by air that has passed through a heating element.

ESR in clinical analysis

Normal reference values for the Westergren ESR method are ≤15 mm for men and ≤20 mm for women. The ESR increases slightly with age with the highest values found in 65-74 years of age. Reference values should be established locally and it is recommended to establish a reference value for each decade of adult life. The probability for disease becomes significant when ESR >50 mm.

Mean and upper limits of Normal (CLSI. 2011)

ESR			Upper Limit of Normal	
Age (years)	mean Male	mean 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
61-70	5,6	9,4	< 12,7	< 20,2
over 70	5,6	10,1	< 30	< 35

Physiological and clinical factors that increase ESR

ESR values are higher for women than for men and increase progressively with age. Pregnancy also increases ESR.

During acute phase reactions, macromolecular plasma proteins, particularly fibrinogen, are produced that decrease the negative charges between erythrocytes and thereby encourage rouleaux formation.

Paraproteins are positively charged molecules that are abundantly present in multiple myeloma and Waldenström's macroglobulinemia patients. Like fibrinogen, paraproteins decrease the negative charges between erythrocytes and increase rouleaux formation. As described earlier the aggregation based ESR tests (Test-1 and iSED) typically miss detecting these disorders, see paragraph above on "Aggregation versus Sedimentation". (Raijmakers, Kuijper, Bakkeren, & Vader, 2008)

High protein concentrations increase plasma viscosity, which slows down the fall rate and thus ESR. However, the effects of fibrinogen and paraproteins on the negative charges between erythrocytes and rouleaux formation far outweigh the effect of increased plasma viscosity, resulting in a strong net increase of ESR.

In anemia, erythrocyte numbers are reduced, which increases rouleaux formation. In addition, the reduced hematocrit affects the velocity of the upward plasma current so that erythrocyte aggregates fall faster.

In macrocytosis, erythrocytes have a shape with a small surface-to-volume ratio, which leads to a higher sedimentation rate.
(Saadeh, 1998) (Bridgen, 1999)

Physiological and clinical factors that decrease ESR

Polycythemia is characterized by an increased proportion of erythrocytes in the blood, which artificially lowers the ESR. Polycythemia can be caused by increased numbers of erythrocytes or by a decrease in plasma volume.

Erythrocyte abnormalities can affect aggregation, rouleaux formation and fall rate. Erythrocytes with irregular or small shapes tend to settle slower and decrease ESR.

A decrease in plasma proteins, especially of fibrinogen and paraproteins, decreases ESR. (Saadeh, 1998) (Bridgen, 1999)

Clinical interpretation of ESR

ESR is a sensitive, non-specific marker of inflammation and is, in combination with clinical history and physical examination, being used as a "general physical condition" marker. There is a linear correlation between fibrinogen levels in blood and ESR readings, so any condition that increases fibrinogen levels, increases ESR. Anemia often occurs in patients with acute or chronic immune activation. Anemia of chronic disease is the second most prevalent after anemia caused by iron deficiency.

Rheumatoid Arthritis (RA) and other autoimmune diseases

ESR measurement is useful in the diagnosis of Rheumatoid Arthritis and the follow-up of RA-patients when combined with other parameters as outlined in the ACR guidelines. However, ESR can be elevated when RA is clinically quiescent and vice versa. ESR is also useful in the follow-up of SLE, but not for inflammatory myopathy or spondyloarthropathy.

Temporal arthritis and polymyalgia rheumatica

An elevated ESR is one of the diagnostic criteria for temporal arthritis and polymyalgia rheumatica. The ESR is almost always elevated in these conditions, in some cases exceeding 100 mm. However, a normal ESR in suspected patients does not rule out diagnosis. If clinical features are present, a temporal artery biopsy is highly recommended, even when ESR is not elevated.

Multiple myeloma

An increased ESR is helpful in diagnosing multiple myeloma, but the final confirmation depends on other criteria (monoclonal spike or serum electrophoresis, marrow plasmacytosis and lytic bone lesions). ESR in benign monoclonal gammopathy is not well studied. ESR measurements should only serve as a guide to disease progression or response to therapy in symptomatic patients.

Other conditions

Clinical studies, often small studies, have suggested possible relevance of ESR levels in different conditions, e.g. bacterial otitis media, acute hematogenous osteomyelitis in children, sickle cell disease, pelvic inflammatory disease, febrile IV drug users, prostate cancer, coronary artery disease and stroke.

An extreme elevation of ESR, defined as >100 mm is indicative for a serious underlying disease, most notably infection, collagen vascular disease, metastatic malignant tumors or renal disease. In most cases, the underlying condition is clinically apparent. In < 2% of patients with an extremely elevated ESR, no obvious cause can be found, but the underlying cause can usually be found in combination with the clinical history, physical examination and other standard laboratory tests. (Saadeh, 1998) (Bridgen, 1999)

Conclusion

Erythrocyte Sedimentation Rate following the gold standard of Westergren is a useful general condition indicator and marker for inflammation. Modern and fully automated instruments have made the ESR test even more accurate and safe in comparison with the manual Westergren version.

The Starrsed automated implementation of the Westergren ESR in addition addresses the many things that might influence the quality of the test result, for example: temperature, stability, dilution, washing and drying of the Westergren tubes and detecting problematic (hemolytic) samples. All these possible quality influencers need to be under control to perform an optimally accurate test.

Several published studies highlight that alternatives to the ICSH and CLSI declared gold standard ESR method that use a test principle that is very different from Westergren, give rise to a large percentage of false negatives and thus a risk of missed diagnoses.

References

Bogdaycioglu, N., Yilmaz, F., Sezer, S., & Oguz, E. (2014). Comparison of iSED and Ves-Matic Cube 200 Erythrocyte Sedimentation Rate Measurements With Westergren Method. *Journal of clinical laboratory analysis*.

Bridgen, M. L. (1999). Clinical Utility of the Erythrocyte Sedimentation Rate. *Am Fam Physician*. Vol. 60, p. 1443-1550

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

Curvers, J., Kooren, J., Laan, M., van Lierop, E., van de Kerkhof, D., S. V., et al. (2010). Evaluation of the Ves-Matic Cube 200 erythrocyte sedimentation method: comparison with Westergren-based methods. *Am J Clin Pathol*.

Dissanayake, D. M. (2006). A rapid method for testing the erythrocyte sedimentation rate. *Journal of Diagnostic Pathology*, 47-51.

Fabry, T. L. (1987). Mechanism of Erythrocyte Aggregation and Sedimentation. *Blood, 1987* (Blood, Vol 70, No5), 1572-1576.

Frankel, S., Reitman, S., & Sonnenwirth, A. (1963). A Text Book on Laboratory Procedures and Their Interpretations. In *Gradwohl Clinical Laboratory Methods and Diagnosis.* The C.V. Mosby Company London.

Grybowski, A., & Sak, J. J. (2011 ;38). Who Discovered the Erythrocyte Sedimentation Rate? *The Journal of Rheumatology*, p. 1521-1522.

Hardeman, M., Levitus, M., Pelliccia, A., & Bouman, A. (2010). Test 1 analyser for determination of ESR. 1. Practical evaluation and comparison with the Westergren technique. *Scand J Clin Lab Invest*. Vol. 70, p.21-25

Jou, J., Lewis, S., Briggs, C., Lee, S., De La Salle, B., & McFadden, S. (2011). ICSH review of the measurement of the erythocyte sedimentation rate. *Int J Lab Hematol. Vol. 33, p.125-132*

Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ. (2017). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. *Int J Lab Hematol. 2017;39(5):448-457. doi:10.1111/ijlh.12693.*

Maas A Van Der, Ende CHM Van Den, Eerd J Van, Fransen J, Broeder AA Den. The use of different methods for rapid determination of the ESR induces DAS28 misclassification in clinical practice. *Clin Exp Rheumatol. 2010;28.*

Madrenas J, P. P. (2005). Giving credit where credit is due: John Hunter and the discovery of erythrocyte sedimentation rate. *The Lancet*, 366, p.2140-2141

Penchas, S., Stern, Z., & Bar-Or, D. (1978). Heparin and the ESR. *Arch Intern Med*. p.1864-1865

Raijmakers, M., Kuijper, P., Bakkeren, D., & Vader, H. (2008). The effect of M-proteins on the erythrocyte sedimentation rate; a comparison between the StarrSed and TEST 1 analyzers. *Ned Tijdschr Klin Chem Labgeneesk*., p. 201-203

Rogers, R. (1994). The development of 30 minute ESR's on the 'StaRRsed' ESR Analyser. *Medical Laboratory World*.

Saadeh, C. (1998). The erythrocyte sedimentation rate: old and new clinical applications. *South Med Journal, p.220-225*

Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med. 2005; 352(10): 1011-1023. doi:10.1056/NEJMra041809.*

Notes

Mechatronics Instruments B.V.
De Corantijn 13
1689 AN Zwaag, The Netherlands
T + 31 229 291 129
sales@rrmechatronics.com

Mechatronics USA LLC
20 Altieri Way, Unit 4
Warwick, RI 02886 USA
T + 1 401 431-6101
salesamericas@rrmechatronics.com