Can we speed up ESR measurement?

The Westergren method versus alternatives

A whitepaper from

ELITechGroup
EMPOWERING IVD

RR Mechatronics
Masters of Measurement

Why we wrote this white paper

Accuracy and reliability of In-vitro diagnostic (IVD) tests have always had a major influence on high quality healthcare. They support medical practitioners in detecting diseases in time and ruling out other serious medical conditions that cause similar symptoms. With this information, patients get the right treatment and medication, which contributes to a speedy recovery and prognosis.

One of the most commonly performed first-line IVD has been the ESR (Erythrocyte Sedimentation Rate). For many decades now, this test is used as a general physical condition marker, to detect inflammation which could indicate an autoimmune disease, an acute or chronic infection, or malignancy. This makes ESR measurement highly useful in the differential diagnosis and or management of, for example, rheumatoid arthritis and multiple myeloma.

ESR can be measured using different methods, of which the Westergren method is considered to be the gold standard by ICSH and CLSI. Understandably, laboratory scientists always seek to improve the way they work and to speed up testing procedures, which explains the emergence of alternative ESR tests that can be performed faster, some of them after only 20 seconds. However, as you will learn from this white paper, we may be able to speed up the way we live, work and travel; we cannot force red blood cells to settle faster than they do.

A range of scientific studies has proven that new alternatives show very different results from the gold standard Westergren method, which means issues are either overlooked or misinterpreted. And while false positives “only” lead to unnecessary and costly follow up, missed diagnoses can have serious consequences.

The different outcomes of Westergren alternatives can pose a dilemma for many laboratory scientists and medical practitioners. Should they choose the alternative ESR in the name of speed, or stick with the Westergren way of measuring ESR? And what are the consequences of this choice?

In this white paper, we will tell you about the historical background of the ESR test, the way it works and the value of the Westergren method. We will explain why it is still the gold standard and method of choice for laboratories that are uncompromising when it comes to providing accurate and reliable results, irrespective of what IVD test is being performed.

Enjoy the reading!

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Key findings

- The erythrocyte sedimentation rate (ESR) is a non-specific marker and is 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.

- ESR measurement is useful in the diagnosis of rheumatoid arthritis, temporal arteritis, polymyalgia rheumatica, multiple myeloma and several autoimmune diseases. Clinical studies have also suggested possible relevance of ESR levels in other conditions.

- The Westergren method as described by the Clinical and Laboratory Standards Institute (CLSI) is the gold standard and was reconfirmed in 2017 as the reference method for ESR measurement by the International Council for Standardization in Hæmatology (ICSH).

- 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.

- Test-1 and iSED are ESR analyzers that produce ESR results 20 seconds after sampling. It takes however approximately 10 minutes before sedimentation starts at a constant rate. This means that the Test-1 and iSED do not actually measure sedimentation, but rather

Key findings

calculates a mathematically derived ESR, leading to a number of false negatives.

- 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”.

- The Starrsed ESR analyzers from RR Mechatronics, MixRate and Excyte analyzers from ELITechGroup are automated ESR analyzers based on the reference Westergren method as recommended by the ICSH and CLSI.

- Automated implementation of the Westergren ESR using instruments such as Starrsed, MixRate and Excyte 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.

Erythrocyte Sedimentation Rate

The erythrocyte sedimentation rate (ESR) is a non-specific marker, used as a general condition indicator. 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)

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

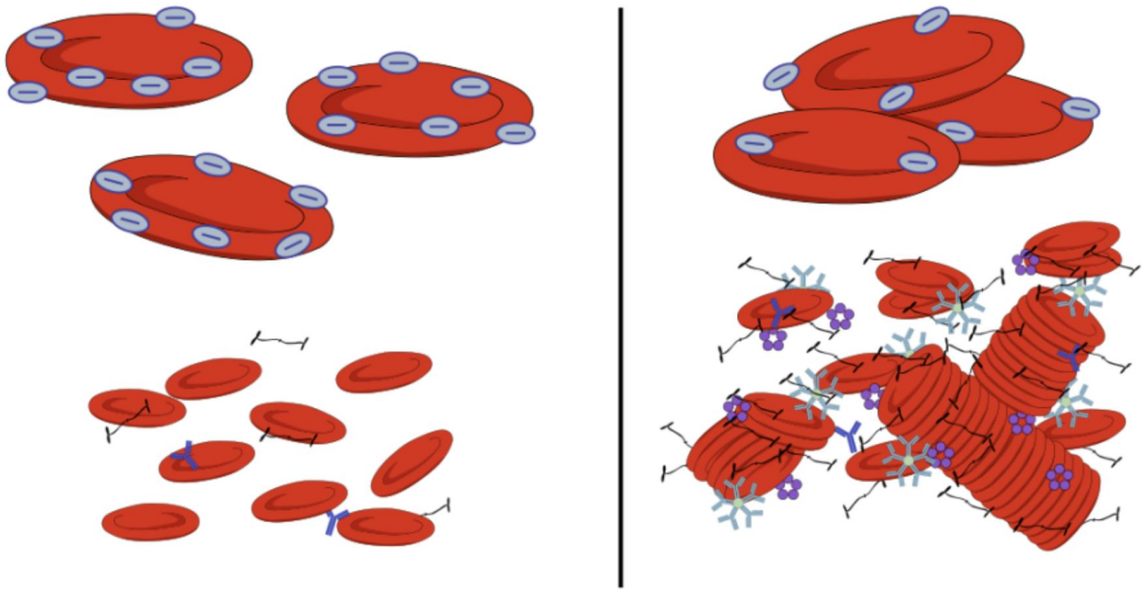

Fig 1: Normal: Negatively charged erythrocytes; low sedimentation rate. Inflammation: less negatively charged erythrocytes; sedimentation occurs, stimulated by all the different factors that increase rouleaux formation (Fibrinogen, CRP, Immunoglobulin).

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

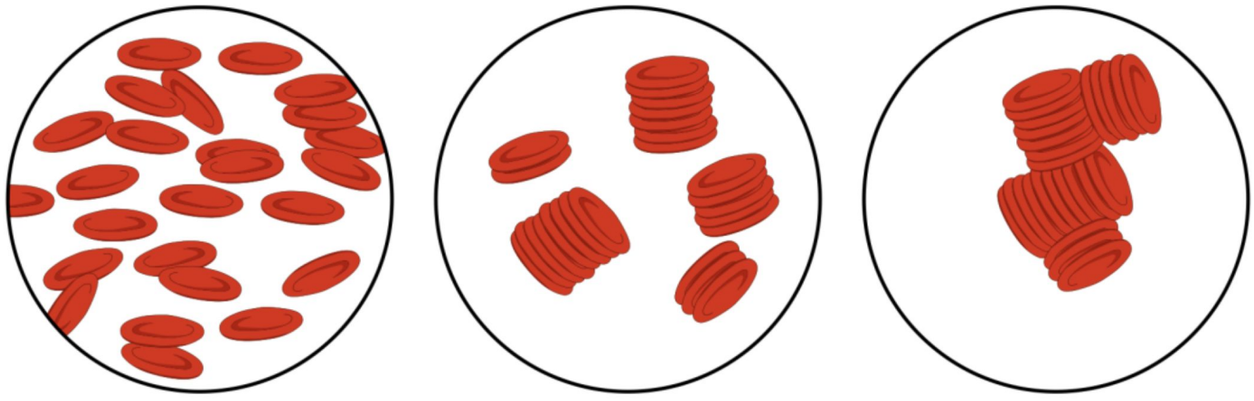

Fig. 2: At low or no flow condition, RBCs adhere side to side and form stacks called rouleaux, followed by end to end connections creating 3D aggregates (the rouleaux formation) (Fabry, 1987)

Clinical interpretation of ESR

As there is a linear correlation between fibrinogen levels in blood and ESR readings, any condition that increases fibrinogen levels, increases ESR. Below, we talk you through some of the conditions for which ESR can be used to assist clinicians in making a correct diagnosis.

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 (Systemic Lupus Erythematosus), but not for inflammatory myopathy or spondyloarthropathy.

Temporal arteritis and polymyalgia rheumatica

An elevated ESR is one of the diagnostic criteria for temporal arteritis 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, meaning ESR measurements should only serve as a guide to disease progression or response to therapy in symptomatic patients.

Other conditions

Clinical studies, often small ones, have suggested possible relevance of ESR levels in different conditions, such as 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 less than 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).

Critical Factors that make an ESR a test that can be relied upon

- Non-hemolyzed blood is anti-coagulated with EDTA at collection
- Blood sample is thoroughly mixed and diluted 4:1 using a sodium citrate solution
- The tube is held in vertical position at a constant temperature (± 1°C) between 18°C and 25°C in an area free from vibrations, drafts and direct sunlight
- Results are interpreted after at least 30 minutes

The Westergren method as the gold standard

In the 1920s, 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) both by the ICSH and the Clinical and Laboratory Standards Institute (CLSI). To this day, the method 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)

Critical factors that make an ESR a reliable test

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

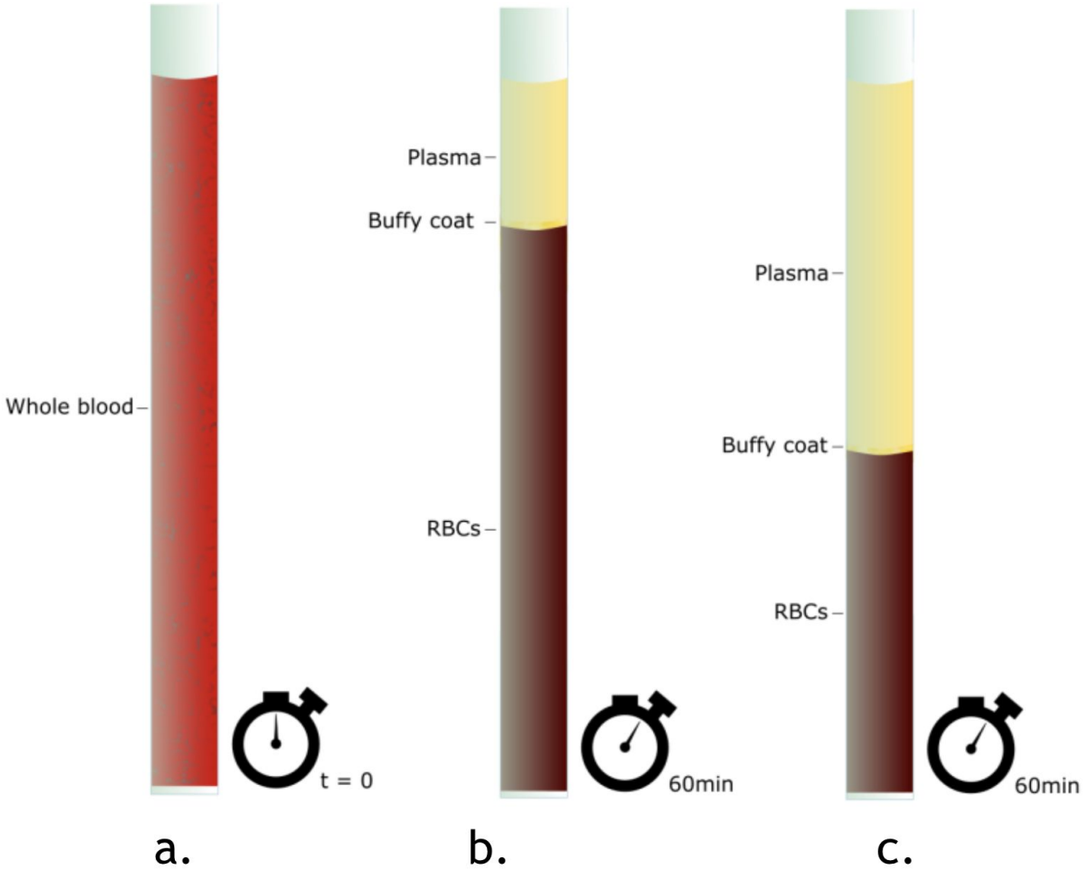

Fig 3:
a. The diluted sample is aspirated and transferred to the Westergren tube.
b. A normal ESR after 60 minutes; <20 mm plasma.
c. An elevated ESR after 60 minutes.

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 and reliability

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.

Comparison between 30 and 60 minutes Westergren method

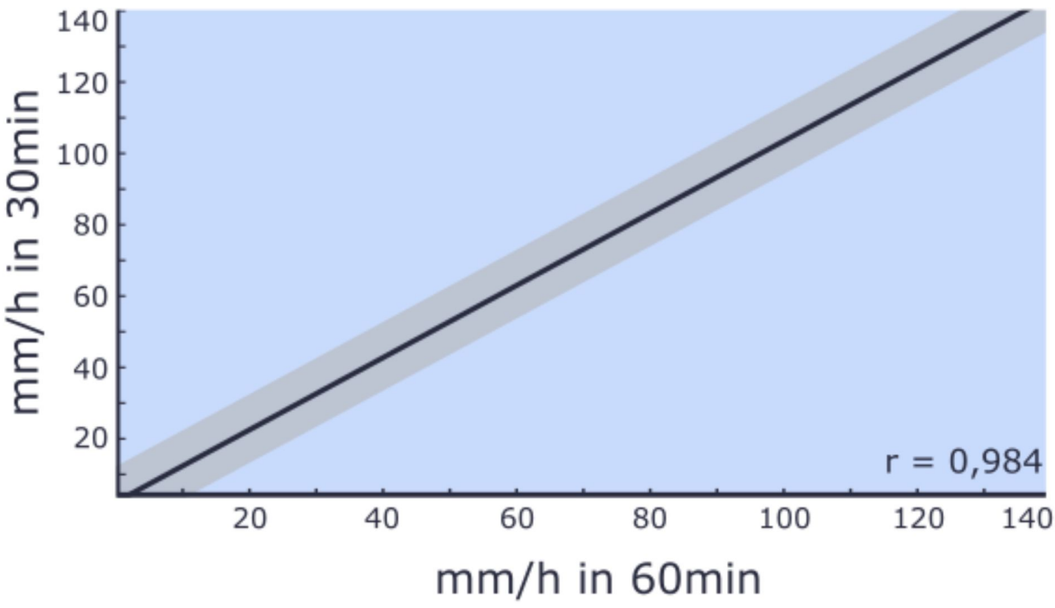

Fig. 4: The 30-minute ESR readings correlate highly with the corresponding 60 minute ESR readings over a wide range of blood samples (correlation coefficient = 0.984).

Second, modern and fully automated instruments like the Starrsed, MixRate and Excyte have made the ESR test even more accurate and safe. Thanks to these instruments, the Westergren ESR implementation can be automated and stabilize the many things that might influence the quality of the test result, such as 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.

Aggregation and sedimentation-based alternatives

In an attempt to speed up ESR readings, several alternatives have been developed to conduct ESR measurements within a shorter time frame. Some examples are Alifax Test-1, Alcor iSED and the Diesse VES-Matic Cube. We will briefly discuss them below.

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 do not actually measure sedimentation, but rather calculate 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.

Like Westergren, the Diesse VES-Matic Cube line of instruments is a sedimentation-based test. To run VES-Matic tests, 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 individual 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

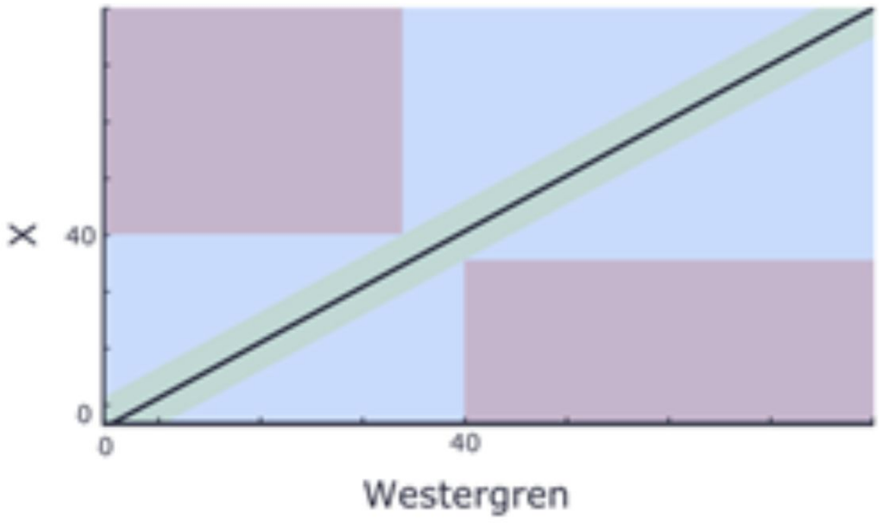

Fig. 5: Results reported into the upper left quadrant are considered normal according to the Westergren gold standard, but high according to method X. These so called "false positives" will lead to additional costs for supplementary testing or unnecessary treatment. Results reported into the lower right 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, such as 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

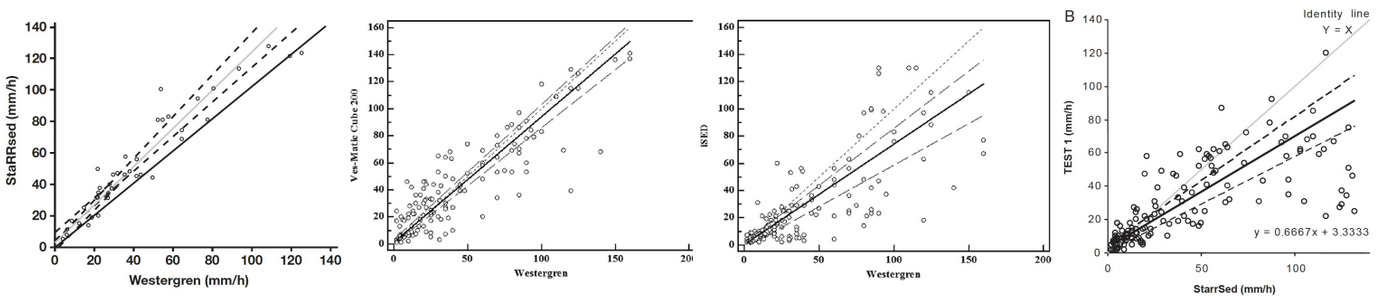

Fig. 6: Higher than 40 on the Westergren scale and lower than 40 on the compared instrument scale are potentially missed diagnoses. (Hardeman, Levitus, Pelliccia, & Bouman, 2010) (Raijmakers, Kuijper, Bakkeren, & Vader, 2008) (Bogdaycioglu, Yilmaz, Sezer, & Oguz, 2014) (Curvers, et al., 2010)

Recent ESR Instrument evaluation articles

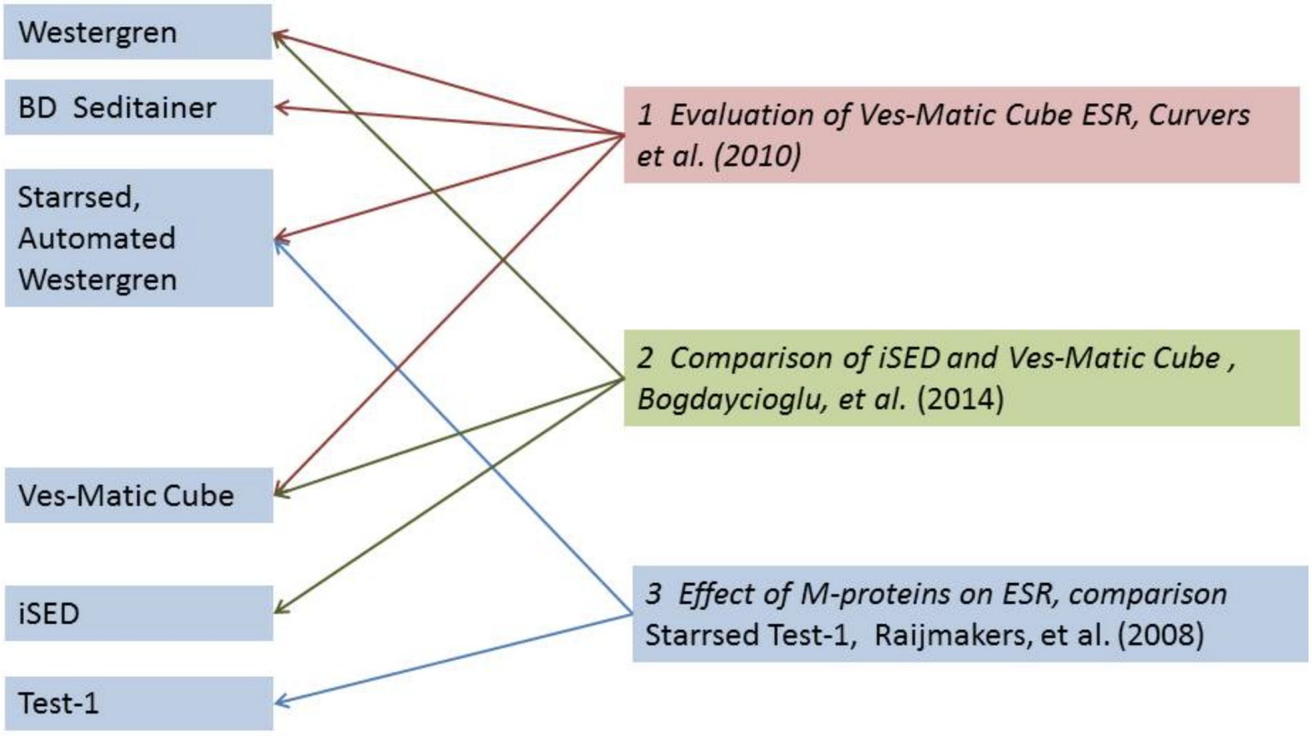

Fig. 7: Different publications evaluating different methods.

According to the ICSH working group, these observations point 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 clinical diagnosis of multiple myeloma or rheumatoid arthritis.

Conclusion

Erythrocyte Sedimentation Rate following the gold standard of Westergren clearly remains a useful general condition indicator and marker for inflammation. Several published studies highlight that alternatives methods using a test principle that is very different from the gold standard Westergren method give rise to a large percentage of false negatives and thus a risk of missed diagnosis.

The reason for this is that Westergren alternatives do not correlate with the Westergren method, which makes them unreliable. Like we explained in the introduction, erythrocyte sedimentation rate is a physical process that needs to be completed before it can be measured. Given the many unsuccessful attempts at speeding up ESR readings, we simply have to accept the fact that some things in life cannot be rushed.

What does this mean to laboratory scientists and medical practitioners? Basically, when you treat healthy people under normal circumstances, alternatives will be comparable to the Westergren method. It is when people get sick that the results start to deviate.

Our advice? Always choose a method that adheres closely to the Westergren method. New instruments such as Starrsed and MixRate perform fully automated ESR readings that give you highly reliable results within a time frame of 30 minutes.

Having read this white paper, we would be very interested in your thoughts on the gold standard. Will you (continue to) work with the Westergren method in the future, or do you need additional information? Let us know, we will be more than happy to share our experience on the matter.

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About ELITechGroup

Our commitment

ELITech Group enables better medical decisions by delivering high-value diagnostic solutions to laboratories operating closer to the patient.
Our mission is to improve patient care by developing market-leading diagnostic products that enable physicians to more rapidly and accurately determine the course of treatment.
ELITechGroup is committed to serve laboratories to a level not delivered today with superior support customers can count on.

How can we help your lab?

As for our part in this story, we can help you research many of the different options that exist to add to the services of your growing lab. Our vast experience and practical knowledge in integrating, maintaining, and innovating has gained the trust of those we serve for decades, and will continue into the future of healthcare, clinical chemistry, and diagnostics.

Please do not hesitate to contact us if you have any questions involving lab expansion, integrated chemistry systems, chemical analyzers, or any of the vast array of diagnostics that we have integrated.

Contact details

Email: info@elitechgroup.com
Phone: +1 435 752 6011

About RR Mechatronics

Masters of Measurement

We are passionate about the invention and development of highly precise analytic instruments. RR Mechatronics, established in The Netherlands in 1986, focuses on medical analytics laboratory instruments. We serve IVD laboratory customers and OEM-partners all over the world.

RR Mechatronics has a track record of thinking in terms of solutions, not problems. We do not have all the answers, but persistently strive to find them in close collaboration with research scientists and our OEM-partners. These partnerships significantly contribute to our high quality work.

For over 25 years we have adhered to the belief in the exponential strength of technological advancement. Our priority is to improve our customers' diagnosis and research operations. In turn, this allows them to treat their patients better. Our commitment to innovation is reflected by the fact that over one in four of our employees works in R&D, also researching solutions outside of the IVD field.

Contact details

Email: sales@rrmechatronics.com
Phone: +31 229 291 129

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