

ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate

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Abstract

Introduction: The gold standard for the determination of the erythrocyte sedimentation rate (ESR) is the Westergren method. Other methods to measure the ESR have become available. They range from modest modifications of the Westergren method to very different methodologies. The ICSH therefore established a Working Group to investigate these new approaches and compile recommendations for their validation and verification.

Methods: A panel of six experts in laboratory hematology examined the peer-reviewed literature and EQA surveys from over 6000 laboratories on four continents performing ESR testing. This information was used to create lists of ESR instrument manufacturers and their methods.

Results: Only 28% of laboratories surveyed used the unmodified Westergren method, while 72% of sites used modified or alternate methods. Results obtained with the new instruments could differ from results obtained with the Westergren method by up to 142%. Different non-Westergren methods showed differences from each other of up to 42%. The new methods were often significantly faster, safer, and less labor-intensive. They reduced costs and often used standard EDTA tubes, eliminating the need for a dedicated ESR tube.

Conclusion: Based on the consensus of the Working Group, recommendations for manufacturers for the validation of new ESR methods were developed. In addition, a list of recommendations for laboratories that are moving to modified or alternate methods was compiled, addressing instrument performance verification and communications of results to clinical users.

KEYWORDS

erythrocyte sedimentation rate, laboratory hematology, laboratory standards, recommendations, westergren

1 | INTRODUCTION

1.1 | History of the erythrocyte sedimentation rate

In many hematology laboratories, the erythrocyte sedimentation rate (ESR) is among the most frequently ordered tests. The procedure was first described in 1894 by Dr. Edmund Biernacki, as

well as independently thereafter by Drs. Hirszfeld, Fähræus, and Westergren.¹ It is based on the principle that the sedimentation of red cells in plasma provides a measure of the level of acute-phase proteins and therefore of inflammation.² While the test is not specific for any particular disease, it remains widely used for its clinical utility in establishing the diagnosis of several diseases, as well as monitoring the activity of selected inflammatory

disorders or therapeutic responses. ESR remains one of the essential prognostic criteria in giant cell arteritis (GCA) and polymyalgia rheumatica.²

1.2 | Overview of the previously published guidelines for performance of the ESR and of the “gold standard” method

From the very beginning, there were significant variations in the methodology used to perform ESR testing.³⁻⁶ The National Committee for Clinical Laboratory Standards (NCCLS; now called Clinical Laboratory Standards Institute [CLSI]) and the International Council for Standardization in Haematology (ICSH) responded by publishing methods for standardizing performance of the ESR.^{3,7-16} The Westergren method was selected as the reference method as it was reliable, reproducible, and sensitive.^{5,6} The defined standardized method recommended the use of blood diluted with trisodium citrate dihydrate and specified the technique, including dimensions and characteristics of the pipettes and how to report the results, namely as millimeter sedimentation after 60 minutes.

In 1977, new documents were published by the ICSH and the NCCLS.^{3,7} Acceptable modifications to the routine method were stated, such as pipettes made of plastic rather than glass, as well as the use of EDTA-anticoagulated blood.

In 1988, both NCCLS and ICSH published new guidelines for quality assurance.¹⁶ In 1993, an ICSH group published new recommendations, stressing the importance of ensuring that measurements obtained in different laboratories were comparable.^{12,14}

Several new methods, some of them automated or semi-automated, became available in 2001. The technical innovations incorporated in these new instruments significantly improved on the existing procedures. Some of the new methods had shorter testing times, others had reduced the biohazards of ESR testing as the samples were aspirated from closed tubes, avoiding exposure of personnel to blood. The CLSI H02-A4 standard covered the new instruments that were available at the time.¹⁴

Despite these efforts, the international standardization and comparability of ESR methods remained unsatisfactory. ICSH and CLSI therefore made new recommendations in 2010 and 2011.^{11,17} The ICSH document recognized that automated methods were routinely used in many laboratories, using diluted or undiluted samples. The reference procedure remained based on the Westergren method. The document stated that all new technologies, instruments, or methodologies had to be evaluated against the Westergren reference method before being introduced into clinical use and that “systems that give the results as the Westergren method with diluted blood at 60 minutes or normalized to 60 minutes are the only ones of clinical value.” It was recommended that manufacturers provide data on the reliability and trueness of any method and instrument, as well as calibration and control procedures. A protocol for evaluation of the routine/working method against the standardized method was also described, clearly indicating the statistical methods that should be used for the comparative evaluation.

This brief summary shows how the procedures published by the ICSH and NCCLS/CLSI, despite some limitations, have for over

40 years provided the guidance needed to ensure comparability of data obtained in different laboratories throughout the world and improved the precision and accuracy of the test.

At present, standardization in this field is facing automation and novel methods to measure the ESR. These pressures are inevitable because of increased workloads, cuts in laboratory personnel and budgets, and the need for closed blood collection tubes to ensure employee safety. The new technologies and instruments address many of these concerns and are therefore attractive to many laboratories. Because of these changes, there is a need for a continuing improvement in the harmonization of the ESR.

1.3 | Aim of this paper

As outlined, standard-setting organizations, including the ICSH, have repeatedly endorsed the Westergren method as the “gold standard” for determining the ESR. Advantages of the Westergren method include high sensitivity, reliability, as well as the availability of a large body of peer-reviewed publications describing clinical applications, limitations, and potential interferences. In 2011, CLSI adopted a standard and ICSH published a review which both list specific details for the reference method for the ESR.^{12,15} The specifics of the methods can be found in these publications and continue to represent the universally accepted gold standard for the ESR. This Working Group fully endorses the continued use of the Westergren method, as described in the last ICSH ESR recommendations, as the gold standard for all ESR measurements. The Working Group also stresses that testing conditions must be adequate, including the appropriate temperature and leveling of the racks, as described in the ICSH and CLSI publications^{11,17}

At the same time, the Working Group recognizes that worldwide many, if not most laboratories have transitioned to the use of either significantly modified versions of the Westergren method (eg, measurements after only 15-30 minutes) or to instruments based on entirely different principles than the Westergren method (eg, centrifugation or photometric rheology). Therefore, the Working Group endeavored to provide a framework of recommendations that will allow clinicians and laboratory leadership to perform an objective assessment about whether and how a particular modified or alternate ESR method can serve the clinical needs of their constituencies.

2 | MATERIALS AND METHODS

A Working Group consisting of the six authors of this study was convened by the ICSH. The members of the Working Group were chosen by the Chair of the ICSH in collaboration with the Chair of the Working Group. Experts had to meet at least one and preferably several of the following five criteria:

- Being responsible for the standardization and quality improvement of laboratory hematology in national or local settings (eg, being responsible for organizing EQA schemes, developing

recommendations in their country/local area).

- Having participated in ICSH and/or CLSI standardization projects.
- Published original peer-reviewed articles and/or edited books on laboratory hematology.
- Were familiar with ISO Standards as well as technical requirements in their own country.
- Geographic diversity; an attempt was made to have as many different areas represented as possible.

Each member of the Working Group reviewed the EQA surveys of his/her geographic area. Instruments with significant market shares in the various geographic areas were then classified as Westergren-based or modified/alternate methods. This allowed an assessment as to the percentage of laboratories using non-Westergren methods (Table 1).

EQA survey data were also analyzed for the presence of differences in results based on the instrumentation used. In surveys where the same EQA material was used for Westergren and non-Westergren methods,

the results obtained with Westergren methods were compared with non-Westergren results. This allowed an assessment of the differences between results obtained with Westergren methods and some of the novel methods. For EQA surveys where different non-Westergren methods were assessed with the same proficiency material, we determined differences between different non-Westergren methods.

The PubMed search engine was used for a literature review to search for “ESR,” “TEST 1,” “STARSED,” “VESMATIC,” as well as “Correlation of ESR with CRP” and “Clinical performance of the ESR for the diagnosis of inflammatory disorders,” concentrating on peer-reviewed articles. The retrieved articles were then used to identify additional publications, which were used to classify instruments into Westergren-based and non-Westergren-based. Approximately 20 relevant papers were retrieved.

Data were shared within the group, and drafts of the conclusions were exchanged until consensus was achieved. Recommendations were based on expert opinion.

TABLE 1 Partial listing of ESR instruments and their methodologies

Instrument	Manufacturer	Methodology
ESR STAT PLUS	HemaTechnologies, Lebanon, NJ	Centrifugation of EDTA-anticoagulated blood. Multiple optical readings of the erythrocyte-plasma interface are used to determine the ESR.
Excyte M	Vital Diagnostics, Lincoln, RI	Samples are diluted with sodium citrate in 120-mm plastic vacuum tubes. Measurement of sedimentation at 30 min, mathematically adjusted to a 1-h Westergren ESR
iSED	Alcor Scientific Inc., Smithfield, RI	Photometric rheology is used to measure the aggregation of red blood cells. Results are correlated with the Westergren method.
Microtest 1	Alifax S.p.A., Polverara, Italy	Utilizes capillary photometric-kinetic technology. Sample is delivered into a capillary tube where it is accelerated via a “stopped-flow” circuit, which causes sedimentation of erythrocytes. Results are transformed to Westergren values and are available within 20 s.
Roller 20 LC	Alifax S.p.A., Polverara, Italy	Utilizes capillary photometric-kinetic technology. Small volume of undiluted EDTA-anticoagulated blood is delivered into a capillary tube where it is accelerated via a “stopped-flow” circuit, which causes sedimentation of erythrocytes. Results are transformed to Westergren values.
Sedimatic 100	Analysis Instrument AB, Bromma, Sweden	Measures the sedimentation of erythrocytes in a vacuum sample tube containing citrate for sample collection and ESR determination
Sediplast ESR	Polymedco, Cortlandt Manor, NY	Manual Westergren and Modified Westergren method
Sedisystem	Becton Dickinson, Meylan Cedex, France	Seditainer ESR tubes are put into a system rack; samples are homogenized. A camera measures the initial cell layer height and the final sedimentation level reading after 20 min. Results are converted by polynomial extrapolation to correlate with conventional Westergren method.
Seditainer	Becton Dickinson Vacutainer Systems, Oxford, UK	Sealed vacuum extraction of blood into a siliconized 100-mm glass tube containing anticoagulant.
Starsted	Mechatronics Manufacturing BV, Zwaag, the Netherlands	Measures ESR in dedicated tubes using whole blood diluted with citrate. Fully closed, automated system. Sedimentation is measured after 30 min and extrapolated to 60 min values.
Streck ESR Auto Plus	Streck, Omaha, NE	Measurement of sedimentation at 30 min, mathematically adjusted to a result that is comparable to a 1-h Westergren ESR
Test 1	Alifax S.p.A., Polverara, Italy	Utilizes capillary photometric-kinetic technology. A small volume of undiluted EDTA-anticoagulated blood is delivered into a capillary tube where it is accelerated via a “stopped-flow” circuit, which causes sedimentation of erythrocytes. Results are transformed to Westergren values.
Vesmatic Cube 200	Diesse Diagnostica Senese, Siena, Italy	Uses standard EDTA tubes; samples are allowed to settle for 20 min, and results are converted to Westergren units.

To complete our survey of changes in ESR testing, we also reviewed trends toward integrating ESR instruments into laboratory automation systems. This was carried out by talking to experienced colleagues and instrument manufacturers and reviewing information about laboratory automation on the Internet.

3 | RESULTS

3.1 | Findings of the literature review

Review of the peer-reviewed literature yielded over 20 original papers on novel ESR instrumentation. Most publications compared the new instruments to the Westergren method. Some of these papers were not fully conclusive, stressing the importance of careful study designs. Other investigators compared new instruments to modified Westergren methods, to each other, or to C-reactive protein (CRP). Interestingly, different authors sometimes arrived at very different conclusions about the clinical usefulness, or lack thereof, of the same methodology.¹⁸⁻²⁷ At least two groups, using different technologies, modified reference ranges to compensate for systemic biases of the instruments they used.^{23,28} In addition, one of these publications also adjusted their ESR reference ranges for the patients' hematocrit.²⁸ One publication presented data that paraproteins had different effects on ESR results depending on the methodology used.²⁹ Van der Maas and co-workers reported that when ESR results obtained with the Westergren method were replaced by an alternate method, the Disease Activity Score 28 (DAS 28), a validated tool to monitor patients with rheumatoid arthritis, misclassified patients.²² All 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.

Comparisons of the ESR with CRP were reported by several groups.³⁰⁻³⁴ Kermani and colleagues reported that the CRP was slightly more sensitive for a positive temporal artery biopsy than the

ESR; however, the difference was minimal.³² A group from Texas found that one in eight patients will have discordant ESR and CRP results.³¹

3.2 | Findings of the review of EQA and other data

We collected EQA and other data from Australia, China, Europe (with separate data sets from Ireland, Italy, and the United Kingdom, as well as from a pan-European survey), Korea, the USA, and Canada (Table 2). A total of 6333 laboratories were represented. 4568 laboratories (72%) used modified or alternate methods for determination of the ESR. Only 1766 laboratories (28%) used the unmodified Westergren method. There was no geographic region that used unmodified Westergren methodology for the majority of their ESR assays, indicating a universal spread of the use of the modified and/or alternate methods. Many EQA surveys used or were in the process of trialing different materials for the various new ESR instruments on the market. For example, the College of American Pathologists (CAP) now offers a general ESR survey for Westergren-based methods, as well as three additional surveys designed specifically for instruments of certain manufacturers that use alternate methods. Many EQA providers, including CAP, use commercial QC materials as raw material, adjusting them for different levels for use as EQA material.

A review of the cumulative results of the surveys indicated that where the same EQA material was used on instruments based on the Westergren method and on non-Westergren-based measurement principles, results often varied significantly (Table 3). Differences were present at both low and high ends of the measurement ranges. In some cases, differences between Westergren and non-Westergren-based methods were higher than 40%; the highest difference observed was 142%. This comparison was based on over 286 sites for the Westergren-based method and 376 sites for the non-Westergren method.

Comparisons between different non-Westergren methods showed differences of over 40 percent. As noted, EQA providers have started to provide different EQA materials to users of various

TABLE 2 Surveys of external quality assessment and vendor data

Country	Total Number of Laboratories Responding	Number of Laboratories Using Westergren or other manual method	Number of Laboratories using Alternate or Modified Methods	Comments
Australia	499	244	255	Separate EQA Module for StaRRsed, another module in trial.
China	729	240	489	Laboratories with certain alternate methods do not participate in EQA.
Europe	418	23	395	Based on EQA
Ireland	57	29	28	Based on EQA
Italy	102	0	102	Based on EQA
Korea	495	Unable to estimate	495	Based on vendor survey
United Kingdom	210	29	182	Based on EQA; three pilot surveys; all methods claimed traceability to Westergren. However, there were differences in results, especially at higher ESR values.
USA and Canada	3823	1201	2622	Based on EQA
TOTAL	6333	1766 (28%)	4568 (72%)	

TABLE 3 (A) Comparisons of EQA results of westergren and non-westergren methods, using the same EQA material for both westergren and non-westergren methods. (B) Comparisons of EQA results of different non-westergren methods, using the same EQA material on all instruments

	Method	Number of participating laboratories	Mean	SD	CV	Maximal difference between methods
(A)						
USA:	Westergren	812	6.5	2.1	32.5	100%
	Non-Westergren	368	13.2	3.0	22.9	
	Westergren	810	42.0	7.5	17.8	80%
	Non-Westergren	371	75.6	8.9	11.8	
	Westergren	286	45.2	6.6	14.6	142%
	Non-Westergren	376	109.4	10.5	9.6	
UK:	Westergren	26	41.1	0.2	22.9	44%
	Non-Westergren	12	59.2	0.5	66.1	
	Westergren	28	45.2	0.2	19.8	48%
	Non-Westergren	16	66.7	0.36	44.0	
(B)						
Italy:	Non-Westergren	7	60.6	6.3	10.4	42%
	Non-Westergren	21	75.4	12.8	17.0	
	Non-Westergren	2	86	42.4	49.3	

non-Westergren-based methods. As these EQA materials are specific for a single method, comparisons of EQA results between different platforms are sometimes not possible.

3.3 | Role of ESR instruments in automated laboratories

Most ESR instruments are stand-alone instruments. However, many laboratories have adopted automation, where tracks transport samples to pre-analytical devices such as centrifuges and decappers, and then to instruments, tube sorters, and storage areas. In addition to stand-alone instruments, ESR instrument manufacturers have therefore started to offer devices that can be connected to automated tracks, making the ESR analyzer an integral parts of laboratory automation. There are three ways an ESR instrument can be connected to an automation track:

- The ESR instrument can be directly connected to an automation line: Examples of this are the Starrsed RL (RR Mechatronics, Zwaag, the Netherlands),³⁵ the Jo Plus (Alifax, Polverara, Padova, Italy), and the Ves Matic Cube 80 (Diesse, Monteriggioni, Siena, Italy) which can be used as either a stand-alone instrument or connected to hematological lines such as the Sysmex XN-9000 with full integration into laboratory automation.
- A similar approach is to transport samples via an automation track to an ESR instrument. A robotic arm, which is part of the ESR instrument, then takes the tube from the track and moves it into the instrument. After aspiration of an aliquot of the sample, it is returned to the track by the robotic arm. This approach is in use in the Starrsed TL (RR Mechatronics, Zwaag, the Netherlands).

- It is possible that manufacturers will integrate rapid ESR methods as part of future CBC testing platforms.

Advantages of integration of ESR technology into automated systems include savings on labor, no need for aliquots and therefore more efficient use of sample volumes, shorter turnaround times, and minimal exposure of laboratory staff to biohazards. Disadvantages include possible higher costs of instrumentation.

4 | DISCUSSION/RECOMMENDATIONS

4.1 | Modified and alternate methods to measure the ESR

As outlined, the traditional Westergren method has been replaced in most laboratories with novel instrumentation. Our surveys indicate that worldwide two-thirds of all laboratories now use modified or alternate ESR test methods for the measurement of the ESR (Table 2). These methods include centrifugation or the use of photometric rheology to measure Rouleaux formation. Results obtained with these diverse approaches can differ significantly from observations obtained with the Westergren method and from each other. In particular, while the Westergren method measures the final length of sedimentation, some of these alternate methods measure the rate of erythrocyte sedimentation, thereby reflecting the name of the test. These methods should be acceptable when they have been appropriately validated, and their results are expressed by comparison with the gold standard. Our review of Proficiency Testing Survey Reports indicates that Westergren-based results usually correlate very well with each other. Modified Westergren methods often use measurements of less

than 60 minutes with mathematical extrapolation to an hour. Such methods correlate reasonably well with Westergren. Some modified Westergren methods use tubes of different length or diameter than endorsed by published recommendations. Other modified methods limit measurements to 15 or 30 minutes. These approaches can show significant differences to the Westergren method at higher values. Finally, instruments based on non-Westergren methodology that have not been validated by the manufacturer as outlined below should not be accepted for clinical use.

In addition to differences in results, some of the new methods do not measure all the ESR phases.¹⁸ It is therefore possible that they will show different susceptibilities to interferences, may be influenced differently by the presence of anemia, or may have different sensitivities and specificities for different disease states (eg, paraproteinemia²⁹ than the traditional Westergren method. Many of these real-life differences, which can have consequences for diagnosis and management, are unlikely to register on EQA surveys, as most of the surveys use commercial material. There are reports in the literature that patients with hypofibrinogenemia may have a lowered ESR, and patients with afibrinogenemia may have an ESR of zero.^{36,37} It is unclear whether the novel methods will similarly reflect low fibrinogen levels.

Reasons for the rapid, worldwide adaptation of these methods include the desire for reduction in the exposure of laboratory personnel to infectious diseases, the ability to use standard EDTA tubes, as well as the faster turnaround times many new technologies offer, often reducing analysis time from one hour to a few seconds. In addition, it should be mentioned that major advantages of the use of EDTA samples are as follows: (i) avoidance of rejections of many samples in everyday practice; (ii) reduction in the blood volume required for hematological tests; and (iii) preservation of the red cells morphology, with maintenance of optimal blood stability.³⁸ The increased automation reduces the probability of human error and increases economic efficiency. Direct interfacing of the instruments with the electronic medical record (EMR) allows error-free, instantaneous data transmission (Table 4). This long list of advantages portends a future with even wider use of the modified and alternate technologies, indicating the urgent need for clear labeling and standardization of the new instruments.

5 | RECOMMENDATIONS

The following recommendations were established based on the expert opinions of the six members of the Working Group. Each member had a primary responsibility in terms of collection and/or analysis of data. After all the data were collected, the Chair combined the contributions into a first draft, which then was circulated repeatedly to the members of the Working Group. Requested changes were communicated by email to all members or by circulating marked-up versions of the manuscript to all the group members. In addition, members conferred at scientific meetings (eg, ISLH, AACC). The final recommendations represent the consensus of all Working Group members.

TABLE 4 Possible advantages of modified and alternate ESR methods

Reduced exposure of laboratory personnel to infectious agents
Ability to use standard EDTA tubes
Reduction in analysis time from 1 h to s
Reduction in probability of human error
Reduction in the amount of labor needed, leading to increased economic efficiency
Ability to interface instrumentation to the EMR, reducing transcription errors and allowing instantaneous communication of results to clinical staff.

5.1 | CLASSIFICATION OF ESR METHODS

The Working Group classifies ESR methods into three categories:

- The Westergren method: This is the gold standard method described in the 2011 ICSH review, without modifications.¹¹
- Modified Westergren methods: These are methods based on the Westergren methodology with some modifications, for example, a shorter assay time and use of no diluent or different diluents than recommended by ICSH.
- Alternate ESR Methods: These are instruments that are not based on the Westergren method. Instead, these devices use novel approaches such as centrifugation or photometric rheology.

5.2 | NEW ICSH RECOMMENDATIONS FOR MODIFIED AND ALTERNATE ESR METHODS

5.2.1 | Manufacturers' Obligations

Standardization (or better harmonization) can be obtained when new technologies are carefully validated against the gold standard method (Westergren). As the modified and alternate methods do not necessarily measure the same pathophysiological processes as the Westergren-based method, the Working Group recommends that these methods be clearly marked by the manufacturers as modified or alternate ESR methods in all promotional materials, package inserts, and user manuals.

In contrast to most other laboratory assays, the ESR does not measure a well-defined analyte with a specific molecular structure, but rather a physicochemical phenomenon, perhaps best described as a "measurand." This means that a true standardization of ESR assays is by definition impossible. A more appropriate term is "gold standard," as represented by the Westergren method.

The following are the Minimal Validation Procedures and Performance Criteria for manufacturers of new modified and alternate ESR methods (Table 5). These criteria are based on previous ICSH documents.

- Accuracy: At least 60 samples, spanning the entire analytical range (2-120 mm), must be analyzed by the Westergren method and the new instrument. Each third of the analytical range should be covered by at least 20 samples. If at all possible, correlation studies should be

TABLE 5 ICSH recommendations for the use of modified and alternate ESR methods

Manufacturers' Obligations	<p>Clearly mark alternate methods as "modified" or "alternate"</p> <p>Determine the closeness of agreement with Westergren methods</p> <p>Determine and indicate the imprecision (reproducibility) of the method</p> <p>List all known interferences and indicate the level at which interference starts as well as the magnitude of the interference</p> <p>Determine age- and gender-specific reference ranges</p> <p>Provide all known information on disease-specific sensitivity and specificity</p>
Performing Laboratories' Obligations:	<p>Perform studies to determine the suitability of the method for their patient population</p> <p>Verify the reference ranges provided by the manufacturer</p> <p>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"</p>

performed with the same method of blood dilution (both in terms of the anticoagulant used and of the level of dilution, if any) for the new and the predicate method. As ESR results are affected by anemia, patient samples used for accuracy studies should have hematocrit results within the reference range. The statistical methods recommended for validations of alternate ESR methods are the coefficient of correlation, Passing-Bablok regression, and the Bland-Altman method.¹¹Correlations and bias should be calculated for both the entire analytical range and for the low, middle, and upper third of the analytical range separately. Correlation coefficients for the three parts of the analytical range should be compared to each other and to the total correlation coefficient. Bias should be constant for the entire analytical range. If these criteria are met, results can be mathematically transformed to corresponding Westergren values. If an alternate method cannot be correlated with the Westergren method, correlation with another alternate method which is validated can be used for method validation.

- Precision:
 - a. Intrarun precision should be determined with at least three patient samples (one each in the low, middle, and high thirds of the analytical range), each analyzed ten times during the same 8-hour period.
 - b. Inter-run precision should be determined with QC material in the normal and abnormal range, analyzed three times a day on five consecutive days.
- Interference studies should be performed for anemia, hemolysis, and lipemia, as well as any other potential interference. Presence or absence of interferences should be noted in the instrument specifications and the standard operating procedures, and if interference is present, the level where interference begins to affect ESR results should be indicated. If appropriate samples from patients with anemia, hemolysis, and lipemia cannot be obtained, spiking of samples or adjustments in hematocrit can be performed.
- The analytical measurement range should be determined by establishing the highest and lowest measurements that correlate with the predicate method.
- Carryover: Potential carryover should be assessed by running patient samples with high and low protein levels and viscosity, in accordance with CLSI document EP10-A3-AMD.³⁹

- Reference Range Studies: Age- and gender-dependent differences in ESR reference ranges have been well documented in the literature.⁴⁰ Age- and gender-specific reference ranges should therefore be determined in accordance with CLSI document EP28-A3c.⁴¹ It is understood that some alternate methods will have reference ranges that may significantly differ from the Westergren method. These values can be mathematically transformed into Westergren units. Alternatively, the ranges obtained in the reference range studies can be used directly, as long as clinical staff is notified via user manuals or package inserts that the results and ranges are different from Westergren results.
- Sensitivity to Fibrinogen: The sensitivity of any new method to increasing amounts of fibrinogen should be determined. A protocol for this procedure has been published by the ICSH in 1992¹² and is reproduced here: A concentrated solution of fibrinogen of approximately 20 g/L is made by dissolving human fibrinogen in distilled water. This is dialyzed overnight against phosphate-buffered saline (PBS; pH 7.4, normo-osmotic) to remove salt content. The fibrinogen concentration of this stock solution is then measured. Five aliquots of 5 mL of normal blood are prepared, and PBS alone or PBS with stock fibrinogen, containing 0, 5, 10, 15, and 20 mg of fibrinogen, is added to each aliquot of normal blood. Calculation of the correlation coefficient and of the slope gives an assessment of the linearity of response and of the sensitivity.

It is the recommendation of this Working Group that only the methods validated according to these well-defined criteria should be considered for routine clinical testing. Manufacturers should clearly state whether the results obtained with their instruments can be traced to the Westergren method.

5.2.2 | User Obligations for modified and alternate ESR methods

- Laboratories that want to introduce modified and alternate ESR methods are obligated to follow all applicable regulatory and institutional requirements. This includes making certain that where

required the instruments have been approved for the local market and meet safety standards.

- Laboratories must confirm the instrument's accuracy by comparing results to their predicate method. At least 30 samples spanning the analytical range of the instrument should be compared. In the absence of an existing predicate method in the laboratory, samples can be sent to another laboratory for accuracy studies. If this becomes necessary, minimization of transport time and maintenance of optimal sample temperature during the transfer must be monitored and kept within acceptable limits. If a laboratory cannot obtain patient samples with high ESR results within a reasonable amount of time, spiking of samples with fibrinogen or paraproteins and analysis with the predicate method and the new system can be performed.
- The analytical measurement range should be confirmed by determining the highest and lowest measurements that the laboratory was able to confirm with the predicate method. This can be performed with the samples used for the accuracy study.
- Carryover: Potential carryover should be assessed for each instrument by the laboratory, to avoid spuriously elevated or low results. This can be performed by analyzing patient samples with high and low protein levels and viscosity, in accordance with CLSI document EP10-A3-AMD.³⁹
- Precision studies should be performed for intrarun and inter-run precision.
 - a. Intrarun precisions should be determined with three patient samples of whole blood (one each in the low, middle, and high thirds of the analytical range), each analyzed ten times during an 8-hour period.
 - b. Inter-run precision should be determined with a normal and an abnormal (elevated) level of QC material, analyzed three times a day for five consecutive days.
- Interferences reported by the manufacturer should be listed in the laboratory's standard operating procedure and shared with customers, as applicable.
- If possible, the laboratory should establish its own reference ranges for the population served by enlisting healthy donors of all age groups. If this is not feasible, the laboratory can verify the reference ranges recommended by the manufacturer, as described in CLSI Guideline EP28-A3c.⁴¹ If necessary, the laboratory may have to adjust for altitude.^{42,43}
- In addition to routine verification studies performed for any new laboratory instrument, laboratories that use modified and alternate ESR methods must, in consultation with clinical staff, perform additional studies to determine the suitability of the new method for their specific patient populations. For example, if a hospital serves a clinic seeing many patients with rheumatic diseases, it is incumbent upon the laboratory to ensure that the ESR method in use is suitable for the clinical needs of these clients. This can be assured by either obtaining clinical performance data from the literature, or by correlating the new method with the predicate method with samples from the patient population in whom the method will be used.

- In addition, the laboratory should issue a change of method notice and should consider initially adding an interpretative comment to every result that summarizes the sensitivity and specificity of the method for various disease states.
- Purchase and use commercial QC material spanning the analytical range of their instruments. If commercial QC material is not available, the procedure described by Plebani and Piva for the use of fresh human whole blood for the daily QC of the ESR can be used.⁴⁴ QC should be run at least once every day that the instrument is in use.
- The laboratory should subscribe to an EQA program specific for its method. If an EQA program suitable for the laboratory's method is not available, regular (two to three times a year) comparison studies with other laboratories should be performed.

6 | CONCLUSIONS

Over 120 years after the first description of the ESR, the clinical relevance of this "imperfect test" has been questioned.⁴⁵ However, the test remains one of the most frequently performed procedures in many hematology laboratories, and novel ways to obtain ESR results safer, faster, cheaper, and with higher accuracy and precision continue to become available. It will be up to the manufacturers, users, and regulators to make sure that the new technologies are chosen, validated and verified, and employed appropriately, for the ultimate benefit of patients, their families, and care providers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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