Comparison of the erythrocyte sedimentation rate measured by the Micro Test 1 Sedimentation Analyzer and the conventional Westergren method

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BACKGROUND: The erythrocyte sedimentation rate (ESR) remains the most widely used laboratory test for monitoring infections, inflammatory diseases and some types of cancer. Several test methods have been developed recently, and as a result, the safety and reliability of ESR testing procedures have improved. The purpose of this study was the comparison of two methods, the traditional manual Westergren method (reference method of the International Committee on Standardization in Hematology) and a new semiautomated technique, the Micro Test 1 for determining the ESR.

SUBJECTS AND METHODS: Blood samples were collected after a night’s fasting from 200 hospitalized and ambulatory patients. Undiluted blood samples anticoagulated with K3 EDTA that had Micro Test 1 values ranging from 2-82 mm/h were used for comparison with the Westergren method.

RESULTS: Linear regression analysis comparing the Micro Test 1 and the reference method yielded satisfactory correlations and regression for samples (r=0.910; P=0.0001; y=4.91+0.86 x; Sy/x=6.85). A Bland-Altman analysis showed no evidence of systematic bias between the Micro Test 1 and the reference method.

CONCLUSION: The Micro Test 1 system was easy to use, had a satisfactory operative practicability, required minimal maintenance, and reduced contact with potential biohazards.

The erythrocyte sedimentation rate (ESR), now more appropriately referred to as the "length of sedimentation reaction in blood (LSRB)" , remains the most widely used laboratory test for monitoring infections, inflammatory diseases and some types of cancer. ESR-related methods have been used since ancient times. Its scientific basis was disclosed in the 18th century, subsequently forgotten and rediscovered in the 19th century. Fahraeus and his scholar Westergren finally popularized the method. The basic factors influencing the sedimentation rate were understood by the early decades of this century and the most satisfactory method of performing the test was introduced by Westergren in 1921. In 1988, the International Committee on Standardization in Hematology (ICSH) (Leuven, Belgium) described an ESR validation procedure as well as a method for producing ESR reference material (ICSH reference method) in the laboratory where it is to be used. The ICSH and the National Committee for Clinical Laboratory Standards (NCCLS), by using the recommended specimen, ethylenediamine tetra acetic acid tripotassium salt (K3 EDTA), which is more reliable than the traditional sodium citrate.1,2 ESR is a nonspecific reaction; it is a measure of the present severity of pathological processes. In general, the ESR is increased in all acute and inflammatory conditions. Variations in the ESR depend on the nature and severity of the process. One of the most important uses of the ESR is in screening for the presence of more or less occult disease and, as such, it is considered a valuable routine procedure.3-7 The ESR still maintains its important role in the diagnosis and follow-up of rheumatoid arthritis and temporal arteritis. It is commonly used as a diagnostic or classification criterion for temporal arteritis and polymyalgia rheumatica, as a prognostic index for monitoring disease activity and for establishing remission in patients. Recently, studies indicate that ESR may be of clinical significance in sickle
cell disease, osteomyelitis, and surprisingly, in non-inflammatory conditions such as stroke, coronary artery disease, and prostate cancer.8,9

The ESR rate measured by the Westergren method is marginally affected by age, race, and blood storage. Despite its importance in many clinical conditions, the ESR should be used only as a clinical guide to aid in the diagnosis, management, and follow-up of these different clinical situations. Hematocrit, fibrinogen concentration and fibrinogen availability (amount of fibrinogen per red blood cell) have effects on erythrocyte sedimentation. Sedimentation is increased in anemia, more so in megaloblastic than in iron-deficiency anemia, and pronounced polycythemia inhibits sedimentation. Sedimentation is also inhibited by variations in red cell shape, e.g., spherocytosis, bacantocytosis, and sickle cell formation.10,11

In recent decades, several new techniques for measuring ESR have been developed and introduced in clinical laboratories. The advantages of the new techniques are to guarantee safety to operators by using automated and closed systems, which automate the measurement itself and optimize the workflow and the use of human resources. The new techniques also create a unique workstation for measuring ESR and performing other hematological tests in a single specimen. We compared the Micro Test 1 semiautomated analyzer (SIRE Analytical Systems, Udine, Italy) and the Westergren erythrocyte sedimentation rate method approved by the ICSH.12

SUBJECTS AND METHODS
In this study, subjects were randomly selected from the entire population of both hospitalized and ambulatory patients. Blood samples were obtained under standardized conditions (collection in the morning after a night’s fasting) and processed for analysis during routine work. Samples were obtained from 200 patients, including 85 men and 115 women. The men were aged 51.27±15.92 (mean±SD) years, range 9-82 years; the women were aged 45.00±16.08 years, range 8-75 years. All blood samples were collected in K3 EDTA vacutainers and in sodium citrate tubes and assayed within 4 hours of venipuncture, and the blood was mixed carefully before mechanical aspiration, according to the ICSH recommendation. Undiluted blood specimens anticoagulated with K3 EDTA were used for analysis. Blood samples with Micro Test 1 ESR values ranging from 2-82 mm/h were selected for comparison studies. The reference technique was the Westergren method, which was performed according to the ICSH recommendation.

The Micro Test 1 is a semi-automated analyzer for measurement of the ESR. The contents of the tubes were mixed by slow rotation for about 3 minutes. This system employs a particular capillary where blood is moved by a special hydrodynamic system. Micro Test 1 requires only 30 microliters of blood and is optimal for pediatric use. The system uses a infrared ray microphotometer with a light wavelength of 950 nm. The electrical pulses, collected by a photodiode detector, are directly correlated to the concentration of erythrocytes present at each capillary level. The pulses, measured per time unit, are then used to delineate the sedimentation curve for each sample by means of a mathematical algorithm. The mean decrease in the signal per time unit, called medium signal, and the square root of the integral signal were transformed to comparable Westergren values by a linear regression model. Improvements were recently made to the original technique. Specifically, the rotation speed was increased from 16 to 60 rpm to achieve a better, more complete mixing of blood. The system operates at a rate of 180 samples per hour in continuous loading, providing a result about every 20 seconds.13

Previous studies have indicated that erythrocyte sedimentation occurs in three phases. Most models of erythrocyte sedimentation are formulated as a sigmoid function, but consider the erythrocyte sedimentation process to consist of three distinct phases: single-cell fall; fall of rouleaux and aggregates, and cell packing. Recently, a piecewise (three-phase) continuous model has been developed. The hematocrit has little influence on either the fall rate of particles in the first phase or the duration of the first phase. This is also true for fibrinogen availability and for fibrinogen concentration at low hematocrit. At high hematocrits the duration of the

Figure 1. Comparison of the Micro Test 1 and the manual Westergren method for measuring ESR by regression analysis (r=0.910, P=0.0001).
first phase increases with fibrinogen concentration. The rate of fall of rouleaux during phase 2 and ESR both decrease exponentially with the hematocrit and increase linearly with fibrinogen concentration. While phase 2 is more closely correlated to fibrinogen availability than to fibrinogen concentration or to hematocrit, this is not the case for ESR. Thus hematocrit, fibrinogen concentration and fibrinogen availability are more important to the velocity of sedimentation in the second phase than to the sedimenting velocity during phase 1 or to the duration of phase 1.

SPSS package 11.00 was used to estimate the linear regression and the correlation between Micro Test 1 and the Westergren method. We computed means and standard deviation. The data from method comparison studies were analyzed by simple least squares linear regression to obtain the y-intercept, the slope, and the SD of the regression line (Sy/x), and Pearson’s correlation coefficient, and the data also were compared by the Altman-Bland analysis.

**RESULTS**

With the MicroTest 1, the ESR was 21.10±16.56 mm/h (mean±SD); with the reference method the ESR was 18.72±17.48 mm/h. Linear regression analysis showed a satisfactory correlation between the two methods ($r=0.910$, $P=0.0001$; $r^2=0.83$; $y=4.91+0.86x$; and $Sy/x=6.85$) (Figure 1). The Bland-Altman data analysis showed no systematic bias and 95% of all samples fell into the narrow 95% limits of agreement ($d−1.96s=2.38−[2×8.87]=−15.00$ and $d+1.96s=2.38+[2×8.87]=+19.76$) (Figure 2).

**DISCUSSION**

The ESR test is one of the most common and traditional laboratory tests in the world. The method is easy to perform and inexpensive and therefore it is used as a routine test for many clinical conditions worldwide. The ESR should be used only as a clinical guide to aid the diagnosis, management, and follow-up of these different clinical situations. In 1988, the ICSH described an ESR validation procedure as well as a method for producing ESR reference material in the laboratory. To determine the factors affecting ESR values, correlations were analyzed between the ESR obtained by the standard Westergren method for red blood cell concentration, haematocrit, and plasma proteins including fibrinogen, albumin and globulins. The ESR has some disadvantages since it requires a large volume of sodium citrate and at least 1 hour of testing time. In this context, several kinds of simple, rapid and safe methods have been developed. These methods offer the advantages of speed, safety, and uniform specimen handling. Systems utilizing sedimentation columns less than 200 mm in length may be less sensitive to changes at higher ESR than the Westergren method. In our study, Micro Test 1 results correlated satisfactorily with the ICSH recommended method. The Bland-Altman analysis

![Figure 2. Bland-Altman plot of ESR values from the Micro Test 1 and the manual Westergren method.](image-url)
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showed no evidence of a systematic bias between the Micro Test 1 and the reference method. The mean difference that we found of 2.38 in our study is nearly the same with the studies performed with Micro Test 1. This difference can be explained with higher values measured with Micro Test 1 at elevated ESR values (>25 mm/h).

In our comparison of Micro Test 1 and the standard method of Westergren, good agreement was obtained. The Micro Test 1 technique produced results similar to those obtained by the Westergren method. The probability of obtaining the same results for all samples using the two different methods to measure the same parameter is unlikely, but it is possible to find the differences between the results of the new and the reference method. If this difference does not affect the interpretation, then one could use the two measurements. In addition, due to its operational characteristics it is a suitable tool for clinical laboratories with a high workload as well as for emergency laboratories. The Micro Test 1 system was easy to use, had a satisfactory operative practicability, required minimal maintenance, and reduced contact with potential biohazards.

REFERENCES

1. International Council for Standardization in Haematology. ICSH recommendation for measurement of erythrocyte sedimentation rate. J Clin Pathol. 1993; 46: 198-203.
2. National Committee for Clinical Laboratory Standards. Methods for the erythrocyte sedimentation rate (ESR) test: approved standard. 3rd ed. Villanova, Pa: National Committee for Clinical Laboratory Standards; 1993. NCCLS document H2-A3.
3. Hertzman A, Evans TI, Sanders KM, Mullinax F. Effects of blood storage on the erythrocyte sedimentation rate. J Rheumatol. 1993; 20: 2178-79.
4. Kaline M, On the temporal development of erythrocyte sedimentation rate using sealed vacuum tubes. Am J Hematol. 1991; 37: 198-9.
5. Van Leeuwen MA, Van Rijswijk MH. Acute phase proteins in the monitoring of inflammatory disorders. Baillieres Clin Rheumatol. 1994; 8 :331-52.
6. Zlonis M. The mystique of the erythrocyte sedimentation rate: A reappraisal of one of the oldest laboratory tests still in use. Clin Lab Med. 1993; 13: 797-800.
7. Saadeh C. The erythrocyte sedimentation rate: old and new clinical applications. South Med J 1998; 3:220-5.
8. Weinstain A, Del Giudice J. The erythrocyte sedimentation rate time honored and tradition bound [Editorial]. J Rheumatol. 1994;21: 1177-8.
9. Brigden M. The erythrocyte sedimentation rate. Postgrad Med 1998; 103:257-74.
10. Bain et al. Some influences on the ESR and fibrinogen levels in healthy subjects. Clin Lab Haematol. 1983; (5): 45-54.
11. ICSH Expert Panel. Guidelines on the selection of laboratory tests for monitoring the acute phase response. J Clin Pathol. 1998; 41: 1203-12.
12. Piebani M, De Toni S, Sanzari MC, Bernard D, Stockreiter E. The TEST 1 automated system : a new method for measuring the erythrocyte sedimentation rate. Am J Clin Path 1998;110: 234-40.
13. Piebani M, Piva E. Erythrocyte sedimentation rate. Am J Clin Pathol 2002; 117:621- 26
14. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986; 1: 307-10.
15. Wolfe F, Michaud K. The clinical and research significance of the erythrocyte sedimentation rate. J Rheumatol 1994; 21: 1177-8.
16. Caswell M, Pike LA, Bull BS, Stuart J. Effect of patient age on tests of the acute phase response. Arch Pathol Lab Med. 1993; 117 (9):6-10.
17. Piva E, Fassina P, Piebani M. Determination of the length of sedimentation reaction (erythrocyte sedimentation rate) in non-anticoagulated blood with the Microtest 1.Clin Chem Lab Med.2002 Jul;40 (7):713-7.