

H2-A4  
ISBN 1-56238-424-4  
ISSN 0273-3099

---

Reference and Selected Procedure for the Erythrocyte Sedimentation  
Rate (ESR) Test; Approved Standard—Fourth Edition

Volume 20 Number 27

John A. Koepke, M.D., Chairholder  
Brian S. Bull, M.D.  
Elkin Simson, M.B., Ch.B., M.Med.  
Onno W. van Assendelft, M.D., Ph.D.



## Reference and Selected Procedure for the Erythrocyte Sedimentation Rate (ESR) Test; Approved Standard—Fourth Edition

### Abstract

This standard is a revision of the third edition approved standard (document H2-A3) published in August 1993. This revision incorporates the most recent recommendations made by the Expert Panel on Blood Rheology of the International Council for Standardization in Haematology (ICSH). The document outlines the necessary details for the performance of a selected (Westergren) method on diluted (1:4) blood specimens and a reference method on undiluted specimens for the determination of the erythrocyte sedimentation rate. Quality assurance and evaluation of other methods to measure the ESR are also described, including procedures for the preparation of a fresh blood reference material for use in the laboratory. Although many see the test as inherently stable and therefore not requiring any additional quality assurance measures, there are available "test kits" that are inadequate. This standard will enable the user of commercial, disposable ESR equipment to ensure that both the test equipment and test procedures are performing adequately.

NCCLS. *Reference and Selected Procedure for the Erythrocyte Sedimentation Rate (ESR) Test; Approved Standard—Fourth Edition*. NCCLS document H2-A4 (ISBN 1-56238-424-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2000.

THE NCCLS consensus process, which is the mechanism for moving a document through two or more levels of review by the healthcare community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of NCCLS documents. Current editions are listed in the *NCCLS Catalog*, which is distributed to member organizations, and to nonmembers on request. If your organization is not a member and would like to become one, and to request a copy of the *NCCLS Catalog*, contact the NCCLS Executive Offices. Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: [exoffice@nccls.org](mailto:exoffice@nccls.org); Website: [www.nccls.org](http://www.nccls.org)

## Contents

Abstract .....	i
Committee Membership.....	v
Active Membership.....	vii
Foreword .....	xv
1 Introduction .....	1
2 Scope.....	1
3 Standard Precautions.....	2
4 Definitions.....	2
5 Precautions and Significant Variables in Performance of the ESR.....	3
5.1 Specimen Collection Variables.....	3
5.2 Time and Temperature of Specimen Storage.....	3
5.3 Equipment Variables.....	3
5.4 Methodologic Variables.....	4
6 Principle.....	4
7 Supplies.....	5
7.1 Pipet.....	5
7.2 Pipet Rack.....	6
8 The ESR Test: Reference Procedure.....	6
8.1 Blood Collection.....	6
8.2 Time of Test.....	6
8.3 Specimen Preparation.....	6
8.4 Packed Cell Volume Adjustment.....	7
8.5 Blood Cell Suspension.....	7
8.6 Handling of the Pipet.....	7
8.7 Reading of the Test.....	7
8.8 Reporting of the Test Results.....	7
8.9 Comparative Values for Routine ESR Methods.....	8
9 The ESR Test: Selected Procedure.....	9
9.1 Blood Specimen.....	9
9.2 Specimen Preparation.....	9
9.3 Handling of the Pipet.....	9
9.4 Reading the Test Results.....	10
9.5 Reporting Test Results.....	10
9.6 Reference Values.....	10
10 Quality Assurance.....	10
10.1 Use of Control Specimens in a Quality Control Program.....	11

**Contents (Continued)**

10.2 Troubleshooting ESR Quality Control Problems..... 11

10.3 Proficiency Testing ..... 12

11 Technical Innovations for ESR Testing ..... 12

Appendix. Protocol for Evaluation of Working ESR Methods Against the Reference Method..... 13

References ..... 14

Summary of Comments and Subcommittee Responses ..... 17

Summary of Delegate Comments and Committee Responses ..... 20

Related NCCLS Publications ..... 24

## Foreword

The erythrocyte sedimentation rate (ESR) test, first described about 70 years ago,<sup>1-3</sup> is one of the most widely performed laboratory tests. The Westergren method<sup>2,3</sup> to measure the ESR has remained essentially unchanged since its inception and was recommended as the method of choice by the International Council (previously Committee) for Standardization in Haematology (ICSH) in 1973<sup>4</sup> and 1977.<sup>5</sup> Although over the years other methods to measure the ESR have been introduced for routine use, e.g., the Wintrobe method<sup>6</sup> and the Zeta Sedimentation Ratio Determination (ZSR),<sup>7</sup> the Westergren method remains the benchmark against which other methods can be, and are, evaluated.

Over the last few years, a number of technical innovations and semiautomated instruments have been introduced that are aimed at eliminating or decreasing the risk of exposure of laboratory workers to potentially infectious material, i.e., blood. The newer procedures are considered less hazardous, primarily because they are either self-contained or use disposable materials, or both. There is a need to examine these innovations, both for comparability of results to previously employed methods and to ensure, on an ongoing basis, the quality of the results. This document provides methods to address both these questions.

Erythrocyte sedimentation remains an only partly understood phenomenon. Three phases can be distinguished in the sedimentation process. The first phase, the lag or aggregation phase, reflects the period in which the individual erythrocytes form rouleaux; there is little sedimentation. During the next, decantation or precipitation phase, the plasma-red cell interface falls more rapidly (increasing sedimentation). In the final or packing phase, the red cell aggregates pile up on the bottom of the tube or container; sedimentation slows down as a result of mutual interference of the closely packed aggregates. Thus, if the descent of the plasma-red cell interface is plotted against time, a typical sigmoid curve is obtained (see Section 6, Figure 1).

The size of the aggregates formed in the lag phase is critical for the outcome of sedimentation. The rates of aggregation and sedimentation are manifestations of the instability of a blood suspension, which is based on a reciprocal effect between the erythrocyte membrane surface and certain plasma proteins; these proteins have been called “agglomerins.” They have a high affinity for the erythrocyte membrane glycoproteins, on the one hand; on the other, they are of sufficient molecular size to form bridges between individual red cells. Fibrinogen, IgM, and alpha<sub>2</sub>-macroglobulin all have “agglomerin” properties<sup>8,9</sup>; fibrinogen cleavage products show a sedimentation activity that decreases with decreasing molecular size.<sup>10</sup> Sedimentation activity of the glycoproteins alpha<sub>1</sub>-acid-glycoprotein, alpha<sub>1</sub>-antitrypsin, ceruloplasmin, and haptoglobin has not been clearly demonstrated, although a positive correlation between the concentration of these acute-phase proteins and erythrocyte sedimentation has been reported. Their concentration, however, rises and falls with that of fibrinogen; thus, this positive correlation may be no more than a manifestation of this parallelism.<sup>8</sup> IgG increases erythrocyte sedimentation only at very high concentrations. Macromolecules not normally found in blood—such as gum arabic, pectin, hydroxyethyl starch, dextrans, gelatin, and hyaluronic acid—may behave as “agglomerins.”<sup>11</sup>

Erythrocytes affect the sedimentation reaction primarily through changes in number and/or shape. Sedimentation is increased in anemia, more so in megaloblastic than in iron-deficiency anemia; pronounced polycythemia inhibits sedimentation. Sedimentation is also inhibited by variations in red cell shape, e.g., spherocytosis, acanthocytosis, and sickle cell formation. Pronounced anisocytosis gives rise to aggregates of different size and to the formation of an “erythrocyte veil” in the supernatant plasma column (“veil sedimentation”).<sup>12,13</sup>

## Foreword (Continued)

Sedimentation is inhibited by increased lysolecithin concentration and by fatty acids; it is inhibited by, e.g., cinchophen, phenylbutazone, sodium salicylate, and thiosemicarbazone.<sup>12,14</sup> Sedimentation inhibition by albumin is disputed.<sup>15</sup>

Erythrocyte sedimentation is a nonspecific reaction; it is a measure of the presence and severity of pathological processes. In general, the ESR is increased in all acute, general infections; in localized, acute, 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.

On occasion, the ESR may be increased where clinical and laboratory evaluation yield negative results. This should nonetheless be regarded as a sign of disease until such time as the physician is fully satisfied that the patient is perfectly well. However, normal values for the ESR have been found in patients with a neoplasm of the liver<sup>16</sup> or with other serious conditions.<sup>17</sup> The ESR may also be useful to differentiate organic disease from functional disorders, or as a guide to the progress of diseases such as rheumatic carditis, rheumatoid arthritis, and certain malignancies, including Hodgkin's disease.

Recently, the ICSH Expert Panel on Blood Rheology was requested to review and update the previously published documents on the ESR.<sup>4,5</sup> Two of the members of the NCCLS Subcommittee on the Erythrocyte Sedimentation Rate also sit on the ICSH panel. The ICSH Panel has reported its recommendations<sup>18</sup> and the conclusions of those recommendations have been freely incorporated into this document. We gratefully acknowledge these cooperative efforts.

## Key Words

Erythrocyte sedimentation, erythrocyte sedimentation rate (ESR) test, quality control, reference procedure, standardized procedure, Westergren pipet

## Reference and Selected Procedure for the Erythrocyte Sedimentation Rate (ESR) Test; Approved Standard—Fourth Edition

### 1 Introduction

The sedimentation of red cells in autologous plasma provides a measure of the acute-phase reaction to inflammation. The term “erythrocyte sedimentation rate” is the traditional term, although a single measurement of the amount of fall of the red cells after 60 minutes is not truly a rate.

Red cell sedimentation is accelerated by an increase in the plasma concentration of so-called “acute-phase proteins,” which are increased in acute tissue damage, chronic inflammation, chronic infection, and pregnancy. The ESR reflects both the increase in certain accelerating proteins, such as fibrinogen and gamma globulins, and the decrease in retarding proteins, such as albumin. This is an advantage for the monitoring of rheumatoid arthritis but decreases the sensitivity and specificity of the test when used for disease screening purposes.<sup>19</sup> Sedimentation is also accelerated in anemia, which may or may not accompany these diseases or conditions. In addition, the ESR is somewhat sensitive to the shape of red cells. For example, red cells such as those seen in thalassemia are broader and thinner than normal and sediment less rapidly than normal erythrocytes.

This document describes a reference procedure for the erythrocyte sedimentation rate (ESR) test, as well as a selected procedure. This selected method is based on the original methodology of Fåhræus<sup>1</sup> and Westergren,<sup>3</sup> which used diluted blood in open-ended, Westergren-type glass pipets of 300-mm length mounted vertically in a rack or stand. The standardized procedure has been verified in studies based on the reference procedure.<sup>18</sup>

Many so-called Westergren pipets, both glass and plastic, have an internal diameter which is less than called for in this document, i.e., less than 2.55 mm. Such pipets have been associated with spurious results, especially in specimens with a packed cell volume (PCV; “hematocrit”) greater than 0.35 (“35%”). Unfortunately, pipets adequate for all blood specimens, including those with higher PCV, are not yet widely available. Therefore, the selected procedure described in this document continues to require dilution of the specimen before measuring the sedimentation “rate.”

A number of technical developments have reduced the biohazard risk of this method, including closed blood collection tubes that are placed upright to function as the ESR tube and are never opened. The simplicity and safety of these new approaches are attractive as the basis of routine laboratory working methods.

The procedures described in this document are an attempt to measure the ESR in a fashion that is not misleadingly influenced by variations in relative erythrocyte volume. The procedures also permit the preparation of a reference material within the laboratory. Such a material, of necessity fresh whole blood, can then be used in the laboratory to ensure that the method routinely in use to determine the ESR, e.g., a routine Westergren method,<sup>18</sup> the Wintrobe method,<sup>6</sup> or the ZSR,<sup>7</sup> provides reliable results.<sup>20,21</sup>

### 2 Scope

ESR procedures cannot be calibrated. The procedures used to determine the ESR are susceptible to a variety of errors. An inadequately performed ESR that produces an incorrect result may not be detected unless some reference material is available in the laboratory where the ESR procedure is being performed. Since the phenomenon of erythrocyte sedimentation is confined to fresh blood and is transient, presently the only feasible way of providing a control material is to specify a method for the production of such

material in the laboratory where it will be used. Because of the nature of the human erythrocyte sedimentation reaction, reference or control materials of the usual type are not available for the ESR test.

This standard specifies the technique and recommends dimensions of the equipment to ensure the precise performance of the ESR test.

If the erythrocyte sedimentation rate test is performed as described in this document, the methods can be used for the following purposes:

- (1) As a routine working method in which the blood specimen is diluted with “physiologic” (0.145 mol/L; 8.5 g/L; “0.85%”) NaCl solution or sodium citrate solution (0.109 mol/L; 32.06 g/L; “3.3%”  $C_6H_5O_7Na_3 \cdot 2 H_2O$ ; CAS number 6132-04-3), four volumes of well-mixed blood to one volume of solution;
- (2) To assign sedimentation “rate” values to fresh patient samples with PCVs of 0.35 or less so that they can serve as quality control specimens for this laboratory test; and/or
- (3) In a suitable protocol, for the evaluation and/or verification of extant as well as newly developed methods for performing the test.

### 3 Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to “standard precautions.” Standard precautions are new guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996; Vol 17;1:53-80), (MMWR 1987;36[suppl 2S]:2S-18S), and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and for recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.

### 4 Definitions<sup>a</sup>

Terms in this document have been used strictly within the limits of the following definitions:

**Reference method (procedure),<sup>22</sup> n** - A clearly and exactly described technique for an analyte which has been shown to provide sufficiently accurate and precise laboratory data for it to be used to assess the validity of other methods for a measurement and for characterizing reference materials; the accuracy of the reference method must be established by comparison with a definitive method, if one exists, and the degree of inaccuracy and imprecision must be stated; **NOTE:** The ESR reference method described in Section 9 is “a clearly and exactly described” technique. There is no definitive method for the determination of the erythrocyte sedimentation rate.

---

<sup>a</sup> Some of these definitions are found in NCCLS document NRSL8—*Terminology and Definitions for Use in NCCLS Documents*. For complete definitions and detailed source information, please refer to the most current edition of that document.

## Related NCCLS Publications\*

- C28-A2**      **How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition (2000).** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.
- H1-A4**      **Evacuated Tubes and Additives for Blood Specimen Collection – Fourth Edition; Approved Standard (1996). *American National Standard.*** This standard contains requirements for blood collection tubes and additives including heparin, EDTA, and sodium citrate.
- H3-A4**      **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fourth Edition (1998).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children. It also includes recommendations on order of draw.
- H7-A3**      **Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard—Third Edition (2000).** This standard describes the standard microhematocrit method for determining packed-cell volume. It also addresses recommended materials and potential sources of error.
- H18-A2**     **Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Second Edition (1999).** This guideline addresses the multiple factors associated with handling and processing specimens, as well as factors that can introduce imprecision or systematic bias into results.
- M29-A**      **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** A consolidation of M29-T2 and I17-P, this document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

---

\* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.