H2-A4
Vol. 20 No. 27

Replaces H2-A3
Vol. 13 No. 8

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

This document provides a description of the principle, materials, and procedure for reference and standardized ESR methods, as well as a procedure to evaluate routine methods, and an outline of quality control programs for the ESR test.

A standard for global application developed through the NCCLS consensus process.

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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.

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

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Suggested Citation

(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.)

Proposed Standard
August 1971
Tentative Standard
March 1972
Approved Standard
October 1974
Revised Tentative Standard
April 1975
Revised Approved Standard
August 1977
Tentative Standard Second Edition
December 1982
Approved Standard Second Edition
August 1988
Approved Standard Third Edition
August 1993
Approved Standard Fourth Edition
December 2000

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

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Contents

Contents (Continued)

Foreword

The erythrocyte sedimentation rate (ESR) test, first described about 70 years ago,[1-3] is one of the most widely performed laboratory tests. The Westergren method[2,3] 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[4] and 1977.[5] Although over the years other methods to measure the ESR have been introduced for routine use, e.g., the Wintrobe method[6] and the Zeta Sedimentation Ratio Determination (ZSR),[7] 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$_2$-macroglobulin all have "agglomerin" properties[8,9]; fibrinogen cleavage products show a sedimentation activity that decreases with decreasing molecular size.[10] Sedimentation activity of the glycoproteins alpha$_1$-acid-glycoprotein, alpha$_1$-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.[8] IgG increases erythrocyte sedimentation only at very high concentrations. Macromolecules not normally found in blood—such as gum arabic, pectin, hydroxy-ethyl starch, dextrans, gelatin, and hyaluronic acid—may behave as "agglomerins."[11]

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").[12,13]

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.[12,14] Sedimentation inhibition by albumin is disputed.[15]

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[16] or with other serious conditions.[17] 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.[4,5] 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[18] 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.[19] 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 Fåhraeus[1] and Westergren,[3] 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.[18]

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,[18] the Wintrobe method,[6] or the ZSR,[7] provides reliable results.[20,21]

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%" $C_6H_5O_7Na_3$ 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[a]

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

Reference method (procedure),[22] *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.

[a] Some of these definitions are found in NCCLS document NRSCL8—*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.

Selected method (procedure),[22] *n* - A method which has been approved by a defined authority as being suitable for routine use, taking account of the limits of its inaccuracy and imprecision in the context of its intended (clinical) purpose, economy of materials and labor, ease of performance, and safety; its validity must be verified by comparability with a reference method; a reference method may be used as a selected method in some instances; when a reference method is not available, or is not practical, in order to ensure harmonization, one selected (routine) method may be designated as a standardized method (procedure); for this, the equipment, reagents, and test procedure must be clearly and exactly specified; **NOTE**: The ESR selected procedure is described in Section 9; a collaborative validation study has been performed.[18]

ICSH has recently proposed that a selected method be renamed "standardized method" and that an ICSH selected method be redefined as "a method which has been recommended for routine use by ICSH on the basis of advice by an ICSH expert panel or other defined authority, after verification of its comparability with a standardized method."

5 Precautions and Significant Variables in Performance of the ESR

5.1 Specimen Collection Variables

Probably the most common difference that impedes interlaboratory comparability of sedimentation rate results is the variety of different specimen collection methods being used. These include the following methods:

(a) Collection of blood in EDTA without any dilution of the specimen;

(b) Collection of blood in special sedimentation rate evacuated tubes that dilute four volumes of blood with one part of citrate solution; and

(c) Collection of blood in the standard coagulation test tubes that dilute nine parts of blood with one part of citrate solution. This practice is not acceptable for determination of the sedimentation rate.

5.2 Time and Temperature of Specimen Storage

The time and temperature of storage of blood specimens before the testing is done is another poorly controlled variable. This is of special concern when laboratories distant from the patient are used for testing. Few studies have been published on this subject, but at least one does indicate that prolonged (i.e., > 4 to 6 hours) specimen retention, particularly if the specimen is not refrigerated, significantly degrades the validity of the test. Refrigerated specimens appear to be satisfactory for testing up to 24 hours, provided they are rewarmed to ambient temperature before testing.[23]

5.3 Equipment Variables

5.3.1 Sedimentation Tubes of Inadequate Diameter

Samples of high ESR and high PCV are the most stringent test of ESR tubes, because erratic plugs of tightly packed red cells cause undue variation in all ESR methods. These plugs variably "shorten" the effective length of the tube and thus decrease the apparent sedimentation rate.

5.3.2 Sedimentation Tubes of Inadequate Material

Certain plastics strongly attract erythrocytes and are thus more susceptible than other materials to the plugging problem referred to in Section 5.3.1. Other tubes (primarily plastic) are coated with mold-

release agents during manufacture or contain plasticizers that interact with blood. In either case, erratic, incorrect results may be produced.

5.3.3 Defective Ancillary Sedimentation Equipment

The degree of vacuum in evacuated tubes designed for ESR testing determines the amount of blood drawn and hence the final dilution that results. Variability here will introduce variability into the final results. Users should take care to allow evacuated tubes to fill to completion.

Some methods utilizing small, disposable, plastic vials for filling of ESR tubes may produce inconsistent results due to inadequate mixing of blood with diluent.

5.4 Methodologic Variables

5.4.1 Errors in the Dilution Step

The dilution step in the Westergren ESR method is needed to prevent variable plugging of the long (200 mm) tube by rapidly sedimenting cells. Even when meticulously performed, the dilution step is still a major contributor to the variability of this ESR method.

5.4.2 Poor Temperature Control

The sedimentation process is substantially influenced by temperature variations, such as those that occur if direct sunlight strikes some tubes but not others, or if air vents from heating/cooling systems are directed at the supporting racks.

5.4.3 Vibration

Particularly if intermittent, vibration can seriously degrade the reproducibility of ESR results.

5.4.4 Verticality

Failure to ensure that the sedimentation tubes or pipets are vertical can cause major problems in ESR results; variation in the angle of the sedimentation tubes or pipets can be problematic in some methods.

6 Principle

The ESR test measures the settling of erythrocytes in autologous human plasma over a specified time period, usually 60 minutes. The reported numerical value is derived from measuring, in millimeters, the distance from the bottom of the surface meniscus to the top of the erythrocyte sediment in a column of anticoagulated blood that has remained perpendicular in a special-purpose pipet for 60 minutes.

If the descent of the plasma-red cell interface is plotted against time, a typical sigmoid curve (Figure 1) results in which three phases can be distinguished.[19] The initial portion of the curve, the lag phase, reflects the period during which the individual erythrocytes form rouleaux. During the second phase, the decantation phase, the plasma-erythrocyte interface falls more rapidly. During the final phase (which may not always be evident within 60 minutes), the cell aggregates pile up on the bottom of the tube or container. The ESR test result, read at 60 minutes, will therefore include varying contributions from the three phases.

The all-important process of erythrocyte rouleaux formation is dependent upon the concentration of the so-called acute phase proteins (e.g., fibrinogen), and, to a lesser degree, the globulins. The erythrocyte

sedimentation process is further affected by the erythrocyte-plasma ratio [the packed cell volume (PCV), also called hematocrit (Hct)]; by the verticality of the sedimentation tube; by the bore of the tube; and by the dilution of the blood sample, i.e., dilution of the plasma factors known to affect the sedimentation of erythrocytes.

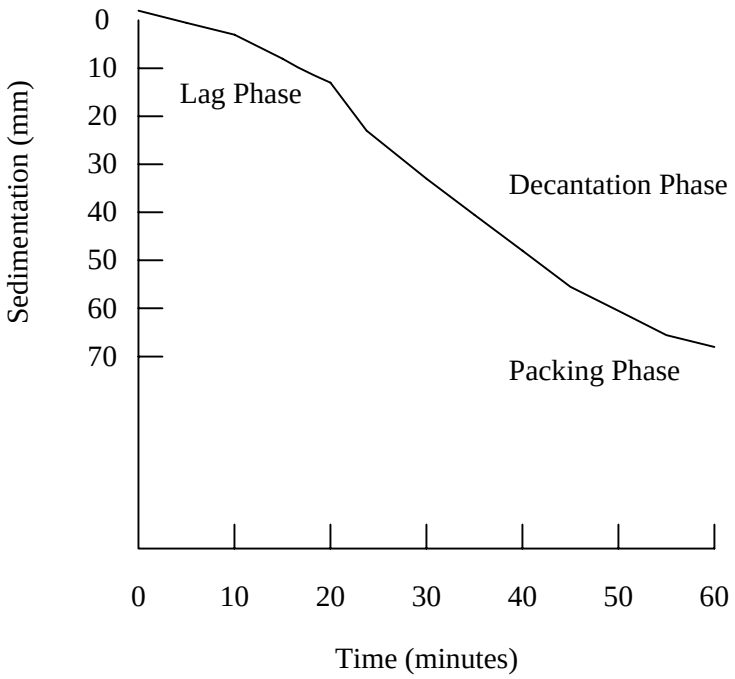

Figure 1. Sigmoid Sedimentation Curve

Because the ESR depends on the ability of erythrocytes to form rouleaux, conditions in which rouleaux formation may be inhibited may be accompanied by normal sedimentation rates. Examples of such conditions include variations in erythrocyte shape, e.g., acanthocytosis, sickle cell formation, spherocytosis, and the presence of abnormal hemoglobins, e.g., hemoglobin C disease, sickling hemoglobins, thalassemia. Pronounced anisocytosis may give rise to aggregates of different sizes and hence to the formation of an erythrocyte veil in the supernatant plasma column ("veil sedimentation").

7 Supplies

7.1 Pipet

The pipet (tube) should be colorless, circular, and of sufficient length to allow for up to 200 mm of red cell sedimentation. A sedimentation scale may be marked on the pipet or adjacently and should comprise clearly marked lines numbered from 200 at the bottom up to 0, in divisions of 1 mm. If separate from the pipet, the scale must be part of a pipet-holding device that ensures precise and reproducible alignment of the pipet and scale. If reading of the pipet is optico-electronic, rather than visual, a marked scale is unnecessary.

The bore of sedimentation pipets for the Westergren ESR was previously recommended to be 2.65 mm ± 0.15 mm.[24] It is now recommended that the pipet diameter be not less than 2.55 mm (no upper limit is specified, except that the volume of blood required should be minimized). The bore should be constant (within 5%) throughout its length and the interior of the pipet should be circular (difference between long and short axes not exceeding 0.1 mm).

The ESR pipet should be disposable. Glass or plastic may be used. Plastic pipets should not show adhesive properties towards blood cells and should not release plasticizers that alter sedimentation. If a mold-release agent is used in the manufacturing process, this must not alter sedimentation.

NOTE: Also compare ASTM[b] standards E923-97: Specification for Glass Westergren Tube, Reusable and E1046-85 (1996): Specification for Glass Westergren Tube, Disposable.

7.2 Pipet Rack

During the test, the pipets must be held motionless in a vertical position. This can be accomplished by use of a rack or stand equipped with an accurate leveling device to ensure that the tubes remain within $\pm 2^{o}$ of the vertical. Presently, only plumb bob racks ensure verticality within these limits. The rack must be constructed so that no leakage of the blood from the pipets can occur.

8 The ESR Test: Reference Procedure

8.1 Blood Collection

Obtain a nonhemolyzed blood specimen by venipuncture in less than 30 seconds. Immediately mix thoroughly with EDTA anticoagulant 3.5 to 5.4 ∝mol (the elementary entity *mol* defined as the anhydrous molecule of ethylenediaminetatraacetic acid, $C_{10}H_{16}N_2O_8$, CAS number 60-00-4, formula weight 292.24), per mL blood. For dipotassium EDTA:anhydrous (K_2EDTA, CAS number 25102-12-9, formula weight 368.4) this equates to 1.4 to 2.0 mg/mL, for disodium EDTA:dihydrate (Na_2EDTA**.**2 H_2O, CAS number 6381-92-6, formula weight 372.2) to 1.4 to 2.0 mg/mL, and for tripotassium EDTA:dihydrate (K_3EDTA**.**2 H_2O, CAS number 65501-24-8, formula weight 442.5) to 1.6 to 2.4 mg/mL. Please refer to NCCLS document H1 – *Evacuated Tubes and Additives for Blood Specimen Collection* for more information on collection tube additives.

The use of evacuated collection systems or blood collection by means of a needle and syringe are both acceptable. Examine the specimen for the presence of small clots (using, for example, an applicator stick) that could invalidate the test results.

8.2 Time of Test

If the specimen is left at ambient (room; 18 to 25 °C) temperature, the test must be set up within four hours. If the specimen is kept at 4 °C, the specimen should return to room temperature and the test should be set up within 12 hours. Stable ESR test results after storage up to 24 hours at 4 °C have, however, been reported.[23]

8.3 Specimen Preparation

Mixing the blood specimen is critically important for reproducibility. For standard tubes (10 to 12 mm x 75 mm, containing 5 mL of blood and with an air bubble comprising at least 20% of the tube volume) there should be a minimum of 12 complete inversions[25] with the air bubble traveling from end-to-end of the tube. Nonstandard tubes, particularly when narrower, may require more than 12 inversions; the required number of inversions should be determined.

[b] ASTM, American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959.

8.4 Packed Cell Volume Adjustment

Determine the PCV and if required, adjust the blood specimen to a PCV of $\leq$ 0.35 by adding autologous plasma.

Adjustment of the PCV is necessary, because many so-called Westergren pipets, both glass and plastic, have an internal diameter less than 2.55 mm (compare Section 7.1). Such pipets have been associated with spurious results in specimens with PCV greater than 0.35.

(1) Divide the specimen into two equal aliquots of about 3.5 mL by using suitable centrifuge tubes.

(2) Centrifuge one of the aliquots at a speed (relative centrifugal "force") and for a sufficient length of time to ensure separation into cells and cell-free plasma.

(3) Add plasma to the second aliquot to obtain a sample with a PCV equal to or less than 0.35. A convenient formula to calculate the least amount of plasma that must be added to 3.5 mL of blood to adjust the PCV is as follows:

$$3.5\left(\frac{PCV\ (as\ \ fraction)}{0.35}\right) - 3.5 = mL\ \ plasma\ required$$

After this manipulation, check that the PCV of the adjusted sample is =0.35.

8.5 Blood Cell Suspension

Before transferring the blood to the Westergren pipet, resuspend the blood cells thoroughly by gently inverting the PCV-adjusted, anticoagulated blood at least 12 times. The inversions should be complete with the air bubble (at least 20% of the tube volume) traveling from end-to-end of the tube. Nonstandard tubes, especially narrow ones, may require more than 12 complete inversions.

8.6 Handling of the Pipet

Using a mechanical suction device, aspirate a bubble-free sample into a clean and dry Westergren pipet, filling exactly to the "0" mark. Place the filled pipet in the vertical position, at 18 to 25 ^{o}C, in an area free from vibrations, drafts, and direct sunlight.

8.7 Reading of the Test

At 60 ± 1 minute, read the distance, in millimeters, from the bottom of the plasma meniscus to the top of the column of sedimented erythrocytes. Be careful not to include any leukocytes (buffy coat) with the erythrocyte column. Record the numerical value. Infrequently, the plasma/red cell interface is so blurred that it is unreadable; the cause for this is unknown.

8.8 Reporting of the Test Results

Express results as the distance, in millimeters, of fall of the top of the red cell column after one hour; only results obtained for the time interval from 0 to 60 minutes are acceptable.

Record the test result as ESR_{ref}, 1 hour = $\underline{x}$ mm.

8.9 Comparative Values for Routine ESR Methods

Limits of acceptable performance of routine (working) ESR methods are given in Table 1.

Table 1. Limits of Acceptable Performance of Working ESR Methods.[18] From the Internal Council for Standardization in Haematology. Recommendations for the measurement of erythrocyte sedimentation rate. *J Clin Pathol*. 1993;46:198-203. Reprinted with permission from the BMJ Publishing Group.

Reference Method*	Working Method Limits†	Reference Method*	Working Method Limits†	Reference Method*	Working Method Limits†
5	1-8	39	14-31	73	38-65
6	1-9	40	15-32	74	39-66
7	1-9	41	15-32	75	40-68
8	1-10	42	16-34	76	40-69
9	2-10	43	17-35	77	41-70
10	2-11	44	17-36	78	42-71
11	2-11	45	18-37	79	43-72
12	3-12	46	18-38	80	44-73
13	3-12	47	19-38	81	45-74
14	3-13	48	20-39	82	45-76
15	3-13	49	20-40	83	46-77
16	4-14	50	21-41	84	47-78
17	4-15	51	22-42	85	48-79
18	4-15	52	22-43	86	49-80
19	5-16	53	23-44	87	50-82
20	5-17	54	24-45	88	51-83
21	6-17	55	24-46	89	52-84
22	6-18	56	25-47	90	53-85
23	6-19	57	26-48	91	53-86
24	7-19	58	26-49	92	54-88
25	7-20	59	27-50	93	55-89
26	8-21	60	28-51	94	56-90
27	8-21	61	29-52	95	57-91
28	9-22	62	29-53	96	58-93
29	9-23	63	30-54	97	59-94
30	10-24	64	31-56	98	60-95
31	10-25	65	32-57	99	61-96
32	11-25	66	32-58	100	62-98
33	11-26	67	33-59	101	63-99
34	12-27	68	34-60	102	64-100
35	12-28	69	35-61	103	65-101
36	13-29	70	35-62	104	66-103
37	13-30	71	36-63	105	67-104
38	14-30	72	37-64		

*Reference method using EDTA undiluted whole blood (PCV = 0.35) in a standard Westergren tube.
†Working method—four parts whole blood to one part citrate. Proposed method valid if 95% of results are within indicated limits.

9 The ESR Test: Selected Procedure

9.1 Blood Specimen

9.1.1 Blood Collection

Obtain a nonhemolyzed blood specimen by venipuncture in less than 30 seconds. Immediately mix thoroughly with EDTA anticoagulant 3.5 to 5.4 ∝mol (the elementary entity *mol* defined as the anhydrous molecule of ethylenediaminetatraacetic acid, $C_{10}H_{16}N_2O_8$, CAS number 60-00-4, formula weight 292.24), per mL blood. For dipotassium EDTA:anhydrous (K_2EDTA, CAS number 25102-12-9, formula weight 368.4) this equates to 1.4 to 2.0 mg/mL, for disodium EDTA:dihydrate (Na_2EDTA**.**2 H_2O, CAS number 6381-92-6, formula weight 372.2) to 1.4 to 2.0 mg/mL, and for tripotassium EDTA:dihydrate (K_3EDTA**.**2 H_2O, CAS number 65501-24-8, formula weight 442.5) to 1.6 to 2.4 mg/mL. Please refer to NCCLS document H1 – *Evacuated Tubes and Additives for Blood Specimen Collection* for more information on collection tube additives.

The use of evacuated collection systems or blood collection by means of a needle and syringe are both acceptable. Examine the specimen for the presence of small clots that could invalidate the test results.

9.1.2 Specimen Storage

If the blood is left at ambient temperature (room; 18 to 25 ^oC), the test should be set up within four hours. If the blood is kept at 4 ^oC, the specimen should return to room temperature and the test should be set up within 12 hours. Stable ESR test results after storage up to 24 hours at 4 ^oC have been reported.[23]

9.2 Specimen Preparation

Mixing the blood specimen is critically important for reproducibility. For standard tubes (10 to 12 mm x 75 mm containing 5 mL blood and with an air bubble comprising at least 20% of the tube volume) there should be a minimum of 12 complete inversions with the air bubble travelling from end-to-end of the tube. Nonstandard tubes, particularly when narrower, may require more than 12 inversions; the required number of inversions should be determined.

Mixing should be continued until immediately before the ESR pipet is filled at the start of the test.

In the selected method, a well-mixed 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%” $C_6H_5O_7Na_3$ 2 H_2O), mixing four volumes of blood with one volume of solution.

9.3 Handling of the Pipet

The pipet should be filled with blood to a height of at least 200 mm. Adjustment of the blood column or scale should be possible to allow correction for slight variation in the nominal volume and ensure an initial reading of zero. During the sedimentation period, and during subsequent disposal, the system must prevent blood spillage or aerosol generation.

Place the filled pipet in the vertical position at ambient temperature (room; 18 to 25^oC) in an area free from vibrations, drafts, and direct sunlight.

9.4 Reading the Test Results

At 60 ± 1 minute, read the distance (in millimeters) from the bottom of the plasma meniscus to the top of the column of sedimented erythrocytes. Be careful not to include any leukocytes (buffy coat) with the erythrocyte column. Record the numerical value. Infrequently, the plasma/red cell interface is so blurred that it is unreadable. The cause for this is not known.

9.5 Reporting Test Results

Express results as the distance, in millimeters, of fall of the top of the red cell column after one hour; only results obtained for the time interval from 0 to 60 minutes are acceptable. Record the test result as the ESR, 1 hour = *x* mm. Note that reporting an ESR result in this manner emphasizes that this test measures a distance after a specified time interval.

9.6 Reference Values

Reference values should be established locally in accordance with recommendations on reference values.[26] For additional information, please refer to the most current edition of NCCLS document C28—*How to Define and Determine Reference Intervals in the Clinical Laboratory*. In view of the progressive rise in ESR with age, separate values should be established for each decade of adult life in men and women. Several other clinical variables influence the ESR and may thus affect physiological reference values, e.g., hemoglobin level, medication, menstrual cycle, pregnancy, and smoking. Table 2 lists reference values for the ESR, which can be used as a guide for the establishment of a local set of reference values.

Table 2. Reference Values for the Westergren ESR Method. Copyright 1984. Novartis. Reprinted with permission from the *Geigy Scientific Tables, Vol. 2, Part 8.* All rights reserved. **NOTE**: All data is given in millimeters at 1 hour.

	Mean ESR			
Age (Yrs.)	Male	Female	Upper Limit of Normal	
			Male	Female
18-30	3.1	5.1	<7.1	<10.7
31-40	3.4	5.6	<7.8	<11.0
41-50	4.6	6.2	<10.6	<13.2
51-60	5.6	9.4	<12.2	<18.6
Over 60	5.3	9.4	<12.7	<20.2

10 Quality Assurance

There are methodologic and equipment variables in the performance of an ESR test to which a quality control program should be sensitive. The availability of control specimens will not, in itself, solve these problems. However, it will, if used in a comprehensive quality assurance program, alert those performing ESR procedures to the existence of problems. Appropriate corrective action can then be taken.

Ideally, an effective quality assurance program should detect such variables as described in Section 5 without placing an undue burden in time or equipment on the laboratory.

Although the ESR procedure cannot be calibrated, it is inherently quite stable. Therefore, unless some change is made in specimen collection equipment or procedure, an ESR method that is in control to start with is likely to remain in control.

Verification of any working (routine) ESR method against the reference method should be performed at a frequency determined by the laboratory's standard operating procedure and particularly when any changes in pipets, personnel, or other variables are introduced. Verification against the reference method, which is performed on undiluted blood, will detect errors in the volume or quality of the diluent and in the adequacy of mixing the diluent with blood in the working method.

10.1 Use of Control Specimens in a Quality Control Program

To obtain blood for such verification, select a patient EDTA-anticoagulated blood specimen of PCV 0.35 or less that contains an adequate residual amount of blood after all other tests are completed and has an elevated ESR value (range 15 to 105 mm) as known from testing or as judged by clinical information or the extent of sedimentation after the sample has stood undisturbed for 30 to 60 minutes. The EDTA-anticoagulated blood specimen in the tube should then be mixed by at least 16 complete inversions. After filling the pipet of the selected method, another aliquot of blood from the same, or from a duplicate, EDTA-anticoagulated blood specimen should be analyzed by the laboratory's working method.

If the blood sample for the working ESR method was taken by venipuncture directly into a citrate or other diluted anticoagulant-containing ESR tube, or if such a dilution was performed in the laboratory, the blood sample for the reference ESR should be taken from a separate EDTA-anticoagulated blood specimen without dilution. This sample may be available if a routine blood count was also requested. This blood sample should have an ESR value between 15 to 105 mm and a PCV of 0.35 or less. A PCV less than 0.35 can also be obtained by following the technique described in Section 8.4. After 60 ± 1 minute, both the reference and the working methods are read. The result of the reference procedure is then translated into the expected result for the working ESR method. Differences from the expected results are tabulated as illustrated in Table 1. For example, if the ESR_{ref} reading is 33, the expected result on the same sample would be 18 (outer limits 11 to 26) with the working method.

10.2 Troubleshooting ESR Quality Control Problems

If the entries in the quality control table are in plus or minus millimeters between the expected and the observed ESR results, the sum of these differences over time should be zero. Interpretation of the quality control results is simply a matter of determining the extent of the scatter in the entries and whether or not a consistent bias exists.

(a) Tubes that are too narrow will bias the results, with the routine method being consistently lower than expected. Also, there will often be an increase in variability.

(b) Vibration, increased temperature, or tilted tubes will cause a positive bias in the routine method. Reproducibility may or may not be degraded.

(c) Difficulty with the dilution step will increase the variability of the expected/observed comparison. If the wrong dilution is being used consistently, then there will be a consistent bias as well.

(d) If the results of the routine method are consistently outside the outer limits given in Table 1 (excessive scatter, consistent bias), the method is unsatisfactory for clinical use. Each component and all procedures employed should be scrutinized and, if necessary, replaced until the method is once more performing satisfactorily.

10.3 Proficiency Testing

Enrollment in an interlaboratory ESR proficiency testing program is also recommended.

11 Technical Innovations for ESR Testing

In recent years, a variety of sedimentation rate test methods have been developed which improves the technical, as well as the biohazardous, aspects of the testing procedure. These methods include micromethods and devices to aid in reading the red cell-plasma interface at the end of the testing period. Other innovations, such as filling tubes without exposure to the blood specimen, have been designed to control biohazards. A number of disposable ESR systems are also presently marketed.

Automated and semiautomated instruments, some of which shorten the testing time, have been developed. Carefully performed comparisons of the system against the reference procedure are required to confirm the accuracy of these methods. (See the appendix.) Refer to the published evaluations of a number of these systems as summarized in Table 3.

All of these methods require a reliable method for calibration and control. Most include comparisons of the proposed method with the reference procedure, or a variation thereof. Test specimens have included a reasonable number of normal, as well as abnormal, ESR specimens.

Table 3. Technical Innovations for ESR Testing

Manual Systems		
Name	**Manufacturer**	**Evaluation**
Dispette 2	Guest	Niejadlik and Englehardt [27]
Seditainer	Becton Dickinson	Berg,[28] Bridgen and Page,[29] Hurd and Knight,[30] Patton et al.,[31] Short et al.[32]
Automated and Semiautomated Systems		
Name	**Manufacturer**	**Evaluation**
B-ESR	HemoCue	Brunborn and Martensson [33]
Sedimatic 8* and 100	Analys Instrument AB	Kallner,[34] Wendland et al. [35]
StaRRsed	Mechatronics R&R	Rogers [36]
Test 1	Sire Analytic Systems	Plebani et al. [37]
Mini-Ves Ves-Matic 20	Diesse	Bridgen and Page,[29] Koepke et al.,[38] Wendland et al.[35]
Systems Without Peer-Reviewed Journal Evaluations		
Name		**Manufacturer**
EsrT		Labdi KB
SediRate		Globe
Sediplus, S-Sedivette		Sarstedt
Sediplus S100, S-Sedivette S200		Sarstedt
Sediplus S2000		Desaga; Sarstedt

*Also marketed as ESR-8 by Streck Laboratories, Inc.

NOTE: Table 3 includes products known to NCCLS at the time this document was published, but it is not all inclusive. NCCLS has not evaluated the listed products; inclusion of products and/or vendors on the list does not constitute endorsement by NCCLS.

NOTE: Also compare Erythrocyte Sedimentation Rate, Survey sets ESR-A, ESR-B, Participant Summaries. College of American Pathologists, Northfield, IL, 1999.

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

For this evaluation, the ESR equipment that is the subject of the evaluation will be referred to as the "test system." The reference/selected method is as described in this document. It uses Westergren-type glass tubes without anticoagulant diluent.

Technical Assessment

(1) Fresh human blood specimens are collected directly into the specified containers of anticoagulant and/or diluent according to the manufacturer's instructions. Specimens should cover the range of sedimentation results from 15 to 105 mm, with approximately the same number of specimens in each quartile. Specimens with a PCV greater than 0.35 should undergo hematocrit reduction as described in Section 8.3; specimens with a PCV equal to or less than 0.35 may be used without further modification. Blood for ESR testing should be stored at ambient temperature (18 to 25 ^{o}C) until tested, and the tests should commence within four hours of collection. If the test system does not incorporate an automatic mixing device, the specimens should be mixed as specified for the selected method (e.g., at least 12 complete inversions for a 10- to 12-mm x 75-mm blood container and more inversions if the inner diameter of the container is smaller).

(2) Precision should be measured on replicate measurements (ten if possible) of a specimen from each quartile. The precision of the reference or selected method should be determined in a similar manner for comparative purposes.

(3) Comparability between the test system and the reference or selected method should be tested in parallel on at least 100 samples from patients with a wide variety of diseases and with ESR results distributed evenly over a range of 15 to 105 mm. Occasional blood samples may fail to give a clear plasma-erythrocyte interface after sedimentation; if this occurs in either the test system or reference/selected method, the pair of values should be eliminated from the data set.

If the blood specimens for the test system are diluted (e.g., four volumes of blood plus one volume of saline or citrate), verification of comparability with the reference method may be determined from Table 1, which already incorporates correction for dilution. Validation is achieved if 95% of the test system results fall within the limits shown in Table 1. Paired results should be plotted on linear graph paper, with differences of the test system ESR from the reference ESR plotted on the vertical axis.[39]

Use of undiluted blood for routine ESR determinations in the clinical laboratory is not recommended because of general unavailability of sedimentation tubes with an inner diameter that is sufficiently large to analyze clinical samples with PCV $\geq$ 0.35.

If the data are analyzed by paired t-test statistics, the values for mean differences, SD of differences, t, and significance (P) should be included in the report.

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NCCLS consensus procedures include an appeals process that is described in detail in Section 9.0 of the Administrative Procedures. For further information, contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Subcommittee Responses

H2-A3: *Methods for the Erythrocyte Sedimentation Rate (ESR) Test—Third Edition; Approved Standard*

General

1. In future revisions of this document, it would be helpful to mention automated ESR technology, its advantages and disadvantages.

   The working group has included a section on Technical Innovations for ESR Testing (see Section 11).

2. Some attention should be given to automated methods in the form of an algorithm for converting reduced time intervals into standard readings. The committee might consider asking the Expert Panel on Rheology to help in developing such an algorithm. Section 3 on Definitions and Section 4 on Precautions and Significant Variables in Performance of the ESR may be the appropriate sections under which to address the automated instrument methodologies.

   The working group does not know of any such algorithm. With experience over the years with many variants of the ESR test, the working group does not believe it possible to develop such an algorithm.

3. The following reference was deleted in the second edition revision of this document.

   "Dawson JD. The E.S.R. in a new dress. *Brit Med Journal.* 1960: 1697-1704.

   As this article provides significant historical perspective for the current ESR test methodology, the reference should be reinstated in the next revision of the document.

   This reference refers primarily to suspension techniques to ensure that ESR tubes are kept in a vertical position. Less cumbersome, adequate alternative techniques are now available.

Section 7.1.1 (now Section 8.1 and Section 9.1)

4. Should the concentration and/or amount/volume of blood of the EDTA be specified?

   Yes, the user is referred to Section 8.1 on Blood Collection for the Reference ESR Method and to Section 9.1 on Blood Collection for the ESR Selected Method.

5. How does one examine the specimen for small clots? If this is not possible, the statement should be deleted.

   Applicator sticks may be used to examine a specimen for the presence of small clots.

Section 7.1.2 (now Section 9.1.2)

6. Section 7.1.2 states that if blood has been stored at 18 to 25 degrees C, the testing must be performed within four hours. Could documentation be provided to support this statement, or is it a "misprint?"

   Between 1983 and 1993, members of the working group have focused on various aspects of ESR testing, including specimen collection and stability. In the course of these studies, it became evident that specimen storage could be extended safely. The user, however, may wish to validate the recommendations by comparing immediate versus four-hour, twelve-hour, and longer storage periods at various temperatures.

   Note that refrigerated specimens should be returned to ambient temperature before testing, because blood viscosity increases rapidly as temperature decreases.

Section 7.4 (now Section 9.4)

7. Several manufacturers are currently marketing automated methods for the ESR. One is a modified 30-minute procedure reading by means of infrared sensors, another is a modified 20-minute procedure utilizing photometry.

   Section 7.4 states that results are to be read at 60 minutes, plus or minus one minute and expressed as the distance, in mm, of fall of the top of the red cell column after one hour. The Sigmoid Sedimentation Curve (Figure 1), upon which this definition is based, shows that the ESR would be incomplete at 20- to 30-minute time intervals. Furthermore, Appendix 1 does not address a reduced time interval.

   The working group agrees. Section 11 on Technical Innovations and several references have been added to address automated ESR methods.

Section 7.6 (now Section 9.6)

8. Is a pipette of bore size greater than 2.55 mm used to establish reference values for Westergren ESR? Is the PCV adjusted?

   The subcommittee recommends that the pipet diameter be not less than 2.55 mm. Adjustment of the PCV is recommended. Users are referred to Sections 7.1 and 8.4 respectively for additional information.

9. Is it critical to establish a reference range for each decade of adult life since the ESR test is not a specific and sensitive test?

   Yes. As an individual ages, the average value for the ESR normally rises until it exceeds the upper end of the reference interval established for healthy, young individuals.

Section 9 (now Section 10)

10. There seems to be a discrepancy between the NCCLS standard and HCFA guidelines. I interpret Section 9, paragraph 3 to indicate that daily quality control is not warranted. However, in July 1991, HCFA established a minimum quality control program for the ESR test, which is defined as performing a patient specimen in duplicate at the start of each shift.

In the United States, this should indeed be done. The working group believes, however, that this will only identify a few of the potential quality assurance failures of the ESR test and that a more comprehensive quality assurance program is important.

Section 9.1 (now Section 10.1)

11. In the example it states, "Convert the difference, in mm/h, to SD (in mm/h; see equation) and plot the results, either negative or positive, on a graph similar to the one shown in Figure 2. How can SDs be negative (if SD=standard deviation)?

The example has been deleted from this document. However, a calculated standard deviation will be positive but can be assigned a plus or minus numerical value as is appropriate from the raw data. The assignment of the correct category (plus or minus) is important for plotting results in a quality assurance chart for visual identification of the presence of bias.

Summary of Delegate Comments and Committee Responses

H2-A4: *Reference and Selected Procedure for the Erythrocyte Sedimentation Rate (ESR) Test; Approved Standard—Fourth Edition*

General

1. I read the sections about specimen collection. No mention is made of systems that draw direct into the citrate solution. This forgoes the need for the EDTA tube and transfer. These new systems should also be addressed.

   This document describes a reference method and a selected procedure (see Section 4 for definitions) for the ESR test. Both procedures require EDTA-anticoagulated blood. These procedures can be used to evaluate "working" (routine) ESR test methods, including methods based on directly drawing specimens into citrate anticoagulant solution or microsedimentation methods. (See Appendix.)

2. The document should contain a guideline as to whether or not an ESR should on sickle cell anemias and patients whose red cells agglutinate at room temperature.

   Section 6, Principle, has been expanded to include a statement of possible interference by erythrocyte shape or erythrocyte content with the ESR test.

3. In the future, information on microsedimentation rates should be added.

   See response to comment 1.

Scope

4. Item (1) states, "As a routine working method...sodium citrate solution (0.109 moI/L; 32.06g/L; "3.3%" C6H607Na3,H20),..."

   It is common for the trisodium citrate anticoagulant to be 106 mol/L. 109 mol/L is less common. In order to avoid unnecessarily excluding effective anticoagulants available in the market place we believe this paragraph should be changed. We recommend it be revised using ISO 6710 (1st edition 1995-08-01, Single-use containers for venous blood specimen collection) as a guide and reference. Annex E of this international standard (copy enclosed) provides for each additive, including trisodium citrate, the nominal amount within a range that shall be used.

   ISO 6710 (1st edition 1995-08-01) states that sodium citrate is used within a range of 0.1 to 0.136 mol/L. The working group considers this range too broad and has followed the International Council for Standardization in Haematology (ICSH) recommendation (*Am J Clin Path.* 1977;66:505-507; *J Clin Path.* 1993;46:198-203) of 0.109 mol/L (32.06 g/L) trisodium citrate:dihydrate (CAS number 6132-04-3; formula weight 294.1).

Section 5.1

5. Item (b) states, "Collection of blood in special sedimentation rate evacuated tubes that dilute four volumes of blood with one part of citrate solution; and..."

There are blood collection systems available that can collect a venous blood sample using an evacuated method or an aspiration principle of collection. One example of such a blood collection system is the Sarstedt S-Sedivette®. Therefore, the word "evacuated" should be deleted from this statement.

This section gives examples of the variety of different specimen collection methods, including, but not limited to, the use of "evacuated" tubes.

6. Item (c) should not be a consideration as it applies only to dilution of EDTA-anticoagulated blood.

In the experience of the working group blood collected for coagulation testing (nine volumes of blood plus one volume of citrate solution) has been erroneously used for the ESR test.

Section 5.3.3

7. We do not believe it is appropriate to identify that blood collection tubes and other items may be defective and cause erroneous results. Defective blood collection tubes are uncommon. The standard should focus on advising laboratory personnel and personnel who collect blood to ensure the correct volume of sample has been drawn.

We believe Section 5.3.3 should be eliminated.

In the experience of the working group variability in the amount of blood drawn into "evacuated" or other types of collection tubes does occur.

Section 8.1

8. Paragraph 2 states, "The use of evacuated collection systems or blood collection by means of a needle and syringe are both acceptable."

The word "evacuated" should be deleted. Also, blood collection by means of a needle and syringe should not be considered acceptable. This method is dangerous and can cause hemolysis and clotting in samples collected.

Routine collection of EDTA-anticoagulated blood generally makes use of "evacuated" tubes. Collection by means of a needle and syringe technique remains acceptable (for example in "countries with limited resources") in spite of the dangers (hemolysis, partial clotting, needle stick injury).

9. Paragraph 1 states, "Immediately mix thoroughly with EDTA anticoagulant, 3.5 to 5.4 Nmol/mL (e.g., K2 EDTA, 1.5 to 2.2 mg/mL; Na2 EDTA, 1,4 to 2.0 mg/mL blood)."

The tripotassium salt of EDTA in the range of 1.2 to 2 mg/mL blood is also an acceptable anticoagulant and is commonly used in Europe and the US. Similar to Section 2, Scope, we recommend this paragraph be revised using ISO 6710 (1st edition 1995-08-01, Single-use containers for venous blood specimen collection) as a guide and reference. Annex E of this international standard (copy enclosed) provides for each additive, including salts of EDTA, the nominal amount within a range that shall be used.

Taking the comments regarding paragraphs 1 and 2 into consideration, we believe paragraph 2 be deleted and paragraph 1 be revised as follows:

"Using a collection system, obtain a nonhemolyzed blood specimen by venipuncture in less than 30 seconds. Immediately mix thoroughly with EDTA anticoagulant and examine the specimen for the presence of clots that could invalidate the test results."

Finally, text should be developed that addresses the EDTA anticoagulant by referencing ISO 6710 (see above).

If the revised text is adopted, the reference to H1 should be deleted.

The working group agrees that K_3EDTA is also an acceptable anticoagulant and has revised Section 8.1 to include this EDTA salt and to clarify the use of substance concentration.

Section 9.1.1

10. Paragraph 2 states, "The use of evacuated collection systems or blood collection by means of a needle and syringe are both acceptable."

    The word "evacuated" should be deleted. Also, blood collection by means of a needle and syringe should not be considered acceptable. This method is dangerous and can cause hemolysis and clotting in samples collected.

    Paragraph 1 states, "Immediately mix thoroughly with EDTA anticoagulant, 3.5 to 5.4 Nmol/mL (e.g., K2 EDTA, 1.5 to 2.2 mg/mL; Na2 EDTA, 1,4 to 2.0 mg/mL blood)."

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    Finally, text should be developed that addresses the EDTA anticoagulant by referencing ISO 6710 (see above).

    If the revised text is adopted, the reference to H1 should be deleted.

    See responses to comments 8 and 9.

Section 11

11. It is very unusual for NCCLS to make reference to manufacturers and the products they supply. This could even be in violation of NCCLS's Administrative Procedures (see Section 4.1.4, Forbidden Discussion Topics). It is important to try and avoid providing any company with a marketing advantage through the use of the standard. Also, to maintain tables like table 3 takes a lot of work to ensure all manufacturers are represented fairly. We strongly recommend it be deleted together with

the final sentence in paragraph 2 that reads "Refer to the published evaluations of a number of these systems as summarized in Table 3." If the table is not removed the following additions should be made:

• *Manual Systems*

Name	Sediplus®
Manufacturer	Sarstedt

Name	S-Sedivette®
Manufacturer	Sarstedt

• *Automated and Semiautomated Systems*

Name	Sediplus® S100
Manufacturer	Sarstedt

Name	S-Sedivette® S200
Manufacturer	Sarstedt

Name	S-Sedivette® S2000
Manufacturer	Sarstedt

Section 11, Technical Innovations for ESR Testing, was added in answer to requests for information on automated ESR methodology (see Summary of Comments and Committee Responses H2-A3, comment 1). The working group summarized innovations in Table 3 in spite of the knowledge that this table would very likely be quickly outdated. The table has been revised to include systems mentioned in the comment.

12. Further information to address the issue of automated instruments and shortened time intervals is needed.

This document describes a reference method and a selected procedure (see Section 4 for definitions) for the ESR test. These procedures can be used to evaluate "working" (routine) methods, e.g., automated test systems, methods incorporating shorter time intervals, etc.

13. Table 3 should include mention of Sediplus Type S2000, Desaga, and Sarstedt technologies.

See response to comment 11.

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.

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