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RECOMMENDATIONS FOR STANDARDIZATION, SAFETY AND QUALITY CONTROL OF ERYTHROCYTE SEDIMENTATION RATE

Prepared on behalf of the World Health Organization

by

John Stuart
Professor of Haematology
Department of Haematology, Medical School,
University of Birmingham, Birmingham, UK

and

S.M. Lewis
Senior Research Fellow and
Director of WHO Collaborating Centre for Haematology
Royal Postgraduate Medical School
Du Cane Road, London, UK

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International Council for Standardization in Haematology

Developments in ESR technology have led to automated test procedures that offer improved precision and a faster ESR result with reduced biohazard risk to the operator. Non-automated but closed ESR systems also offer a significant reduction in biohazard risk. These and other developments in ESR methodology are to be encouraged provided that comparability with the ICSH standardized (or reference) method is maintained. This document describes the criteria for comparability and safety that routine ESR methods should now meet.

1. INTRODUCTION

The erythrocyte sedimentation rate (ESR) test measures the sedimentation of aggregated red cells in autologous plasma. Sedimentation takes place in three stages: a preliminary stage when rouleaux form, a period during which the rouleauxed cells sediment at a constant speed, and a final stage when the rate of sedimentation slows as the cells begin to pack.

The rate of sedimentation depends mainly on the extent of rouleaux; it is increased by a high concentration of fibrinogen and of other acute-phase proteins and immunoglobulins; it is retarded by albumin. The acute-phase proteins are a heterogeneous group of proteins that are synthesized in increased amounts by the liver in response to cytokines (including tumour necrosis factor and interleukin-6) released from monocytes/macrophages at a site of tissue damage. Such damage, sufficient to cause an inflammatory response, may arise from trauma, infection, ischaemia and other factors.

Sedimentation is also accelerated by anaemia so that, unlike plasma viscosity, the ESR test reflects both the hyperproteinaemia and the anaemia of inflammatory disease. When anaemia is caused by an unrelated condition (e.g. deficiency anaemia or blood loss) this will decrease the specificity of the ESR. Attempts have been made to apply correction factors for anaemia, but these are unreliable. The test is also influenced by the shape, size and deformability of the red cells and thus, for example, it is unreliable as an index of illness in sickle cell disease or when there is marked poikilocytosis.

ESR is a non-specific test which can be used to detect a wide range of organic diseases and, as there is a fairly close correlation between the ESR and disease activity, it also provides a means for monitoring progress and response to therapy. It is one of the most widely used clinical laboratory screening tests and is often performed as a clinic procedure near the patient.

The reference method for measuring the ESR, as recommended by the International Council for Standardization in Haematology (ICSH), was based on the method described by Westergren[1] using blood which is diluted (4 vol blood plus 1 vol citrate) and then sedimented in open-ended glass tubing of 300 mm length mounted vertically in a rack or stand. ICSH now recommends measurement using undiluted anticoagulated blood as the basis of a new reference method[2] but recognises that diluted blood will continue to be used for routine methods.

Because of the biohazard risk inherent in performing the Westergren-based method, ICSH has proposed a closed system which is now recommended as an ICSH standardized method[2]. Manufacturers are being encouraged to produce closed systems conforming to the ICSH specifications for the standardized method and which can be used for verification and/or quality control of routine methods that are in day-to-day use for measuring the ESR.

This WHO document describes the new standardized method and makes recommendations on routine methods for measurement of the ESR.

2. MEASUREMENT OF ESR: ICSH STANDARDIZED METHOD

2.1 Sedimentation tube

This must be a colourless glass or plastic tube (or "pipette") more than 200 mm in length with bore not less than 2.55 mm; the bore must be uniform (within 5%) throughout the length of the tube. There must be a scale marked on the tube or separately in mm, extending over the lower 200 mm, and numbered from 200 at the bottom up to 0.

For use the tube must be clean and dry. It should be cleaned in an acetone-water system. Dichromate and the commonly available detergents are not recommended. If the tube is "disposable" it must be supplied clean and dry and the manufacturer should be expected to guarantee that the cleaning procedure does not introduce any factor which may affect its use in the ESR test as compared with glass tubes cleaned by the recommended method.

2.2 Holding device

This is a rack, stand or other apparatus intended to hold the tube motionless in a vertical position. A method to ensure that the tubes are maintained in a vertical position should be an integral part of such a device. It should be constructed so that no leakage of the diluted blood can occur from the filled tube.

2.3 Technique of test

Blood is obtained by clean venepuncture, avoiding contamination with skin cleaning materials. The blood should be of haematocrit 0.35 or less and be anticoagulated with EDTA (1.5 mg/ml blood) with less than 1% dilution when EDTA is in solution, and not further diluted with anticoagulant-diluent citrate solution.

The test must be set up within 4 hours of venepuncture. After careful mixing of the blood (at least 8 complete inversions with the air bubble travelling from end-to-end of the tube) an ESR tube is filled by means of a mechanical suction process which may be automated. The level of blood is adjusted to the "0" mark.

The tube is then placed in the holding device at constant temperature (± 1 °C) within the range 18-25 °C, not exposed to direct sunlight, free from vibration and draughts, and left undisturbed for exactly 60 minutes.

2.4 Expression of measurement

At this time, the distance from the bottom of the surface meniscus to the top of the column of sedimenting red cells (where the full density is apparent), is read in mm and recorded as ESR (undiluted) = X mm. For comparison with routine ESR methods performed on blood diluted with citrate this value requires to be adjusted for lack of dilution (see 3.5).

2.5 Health and safety

Great care must be taken to avoid blood spillage or aerosol spray with resulting risk of contamination to the operator and the surrounding area. Under no circumstances should mouth suction be used. Standard precautions should also be taken in subsequent washing of non-disposable Westergren tubes especially when dealing with blood from "high risk infectivity" patients.

2.6 Application of the ICSH standardized method

The ICSH standardized method is designed to be used for verification and quality control of a routine method as described below (see section 5).

3. ICSH SELECTED ROUTINE METHODS

Developments in ESR methodology have led to the introduction of a variety of ESR tubes that differ in length and diameter from the traditional Westergren tube. Automated methods for mixing the tube contents before sedimentation and for reading the end-point have also been introduced. Laboratories should use, as their routine method, an ESR system that is an ICSH selected method which meets the criteria below.

Laboratories may continue to use ESR tubes of traditional Westergren dimensions but these should be used in a system that accords with health and safety requirements (see 3.6).

3.1 Blood sample

Blood should be obtained by clean venepuncture (manual or vacuum draw) over a maximum period of 30s and without excessive venous stasis. The ESR test should commence within 4 hours of venepuncture. In some vacuum-draw test sytems, when the tube is not opened, the blood may be stored at 4 °C for up to 24 hours before testing but this duration of storage must be validated (see below).

The blood should be diluted either at the time of venepuncture (4 vol blood taken directly into 1 vol of sterile sodium citrate anticoagulant-diluent) or taken first into a primary anticoagulant (EDTA 1.5 mg/ml blood) followed by dilution in the laboratory using sterile sodium citrate (see below).

3.2 Anticoagulant diluent solution

Trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$): 109 mmol/l (range 100-136) aqueous solution (32.08 g/l). The solution is filtered through a sterile membrane (0.22 μm pore size) into a sterile container without preservative. The solution may be stored at 4 °C for several months, but must be discarded if it becomes turbid. For use, it is dispensed in 0.5 ml volumes into sterile specimen containers for the addition of 2 ml blood. If not used immediately, the stoppered containers should be kept at 4 °C, in sealed plastic bags to avoid evaporation. They must not be used if the solution is turbid, or has leaked (a volume line marked on the container will provide a check for the latter).

Immediately before the ESR test is set up, the blood and anticoagulant-diluent must be mixed again by at least 8 manual complete inversions with the air bubble travelling from end-to-end of the tube, or by an automated mixing system whose effectiveness has been validated.

3.3 Sedimentation pipette specification

Pipette dimensions for routine methods are not specified by ICSH, and may or may not be of Westergren dimensions, but they must be validated by comparison of test results with the ICSH standardized method. Details of such validation should be provided by the manufacturer.

Pipettes may be plastic or glass and as far as possible should be disposable. If reused, special attention must be paid to their cleaning with removal of all contaminants and an appropriate verification check made thereafter.

3.4 Pipette holding device

During the test procedure, the pipette must be held motionless. If not held vertical, the angle of the incline should be specified and comparability of results with the ICSH standardized method validated. Pipettes should be protected from direct sunlight, draughts and vibration and maintained at a constant temperature (± 1 °C within range 18-25 °C) for the duration of sedimentation.

3.5 Recording the end-point

Some test systems do not employ the traditional 60 min period of sedimentation. A test reading is sometimes made after 20-30 min sedimentation and normalised to a 60 min result. Test systems may incorporate a mathematical adjustment for initial height of the blood column, haematocrit of the sample, ambient temperature, or duration of sedimentation. The ESR result, whether requiring mathematical correction or not, should be expressed so as to achieve comparability with the ICSH standardized method, values for the latter being adjusted for lack of dilution using the ICSH formula[3]:

ESR mm (diluted) = [ESR mm (undiluted) x 0.86] - 12

See also 5.1.

3.6 Health and safety

It is essential to minimise the biohazard risk to laboratory staff. Under no circumstances should ESR pipettes be filled by mouth suction and during the test procedure it is especially important to avoid blood spillage or aerosol generation. ICSH selected methods should therefore minimise the biohazard risk of the reference method. ICSH and WHO recommend the use of ESR methods in which the blood sample tube and test system remain sealed throughout the procedure and during disposal.

4. REFERENCE VALUES

Values for "normal" ESR have not been given in ICSH publications as they may be influenced by local conditions. A range for the local healthy population should be established nationally or regionally. It should, however, be noted that the range will be influenced by the number of anaemic subjects in a so-called normal population. The ESR may also be influenced by age, sex, menstrual cycle, drugs taken (e.g. corticosteroids, contraceptive pills).

The following data of normal values, derived from several publications, represent a consensus which is, in general, valid for western industrialized countries. In some countries the ESR is frequently elevated in symptom free individuals possibly due to high concentrations of immunoglobulins:

	Mean	SD*	Upper limit of reference ramge
Men aged 17-50	4	3	10
51-60	6	3	12
61-70	6	4	14**
Women aged 17-50	6	3	12
51-60	9	5	19
61-70	10	5	20**

* All expressed as ESR in mm. The 2SD range includes 95% of the healthy population.
** Above 70 years, it is particularly difficult to define and identify a healthy population and determine appropriate reference values.

By the standard Westergren method, the ESR in childhood appears to be the same as for normal men (< 10 mm) with no difference between boys and girls.

Increased ESR is found as a manifestation of a wide spectrum of diseases, mostly related to an increase in acute-phase proteins (see Introduction), and also in normal pregnancy and the puerperium.

The ESR may be especially low (0-1 mm) when there is polycythaemia or severe hypofibrinogenaemia. Apart from these conditions, which will tend to obscure a high ESR, it is unusual to have a false negative result; thus a normal ESR will generally give assurance that inflammatory disease is not present.

5. VERIFICATION AND QUALITY CONTROL OF A ROUTINE METHOD

Verification of the routine method should be performed against the ICSH standardized method. This is usually undertaken by the manufacturer in collaboration with a clinical laboratory. Once in routine use, the routine method should be verified at intervals against the ICSH standardized method as a regular quality control procedure. The frequency of this is determined by the laboratory standard operating procedure but should always be undertaken when there is a change in the routine procedure or a new batch of tubes or stock of citrate is used.

5.1 Comparison of results

As the ICSH standardized method is performed on undiluted blood compared with most routine methods which use diluted blood, it is necessary to apply a correction to the standardized method for lack of dilution[2]. Absence of dilution in the standardized method permits the detection of errors in the volume or quantity of diluent in routine methods so that the ICSH standardized method can be used as a quality control procedure.

The results by the standardized method should be recorded as the sedimentation at 60 min and expressed as ESR (undiluted) = x mm. The ICSH formula[3] (see 3.5) can be used to correct for lack of dilution when comparing the result with routine methods that use diluted blood but a recommended alternative method for verification of the comparison is given in Table 1.

5.2 Micromethods

ESR pipettes that require a reduced draw volume have been introduced by some manufacturers, particularly for paediatric use. Their comparability with the ICSH standardized method should be validated.

6. CONCLUSION

Developments in ESR technology have led to automated test procedures that offer improved precision and a faster ESR result with reduced biohazard risk to the operator. Non-automated but closed ESR systems also offer a significant reduction in biohazard risk. These and other developments in ESR methodology are to be encouraged provided that comparability with the ICSH standardized (or reference) method is maintained. This document describes the criteria for comparability and safety that routine ESR methods should now meet.

REFERENCES

1. International Committee for Standardization in Haematology. Recommendation for measurement of erythrocyte sedimentation rate of human blood. Amer. J. Clin. Path. 1977 ; 66 : 505-507.

2. International Council for Standardization in Haematology (Expert Panel on Blood Rheology). ICSH recommendations for measurement of erythrocyte sedimentation rate. J. Clin. Pathol. 1993 ; 46 : 198-203.

3. International Committee for Standardization in Haematology (Expert Panel on Blood Rheology). Guidelines on selection of laboratory tests for monitoring the acute phase response. J. Clin. Pathol. 1988 ; 41 : 1203-1212.

ACKNOWLEDGEMENT

Table 1 has been reproduced with kind permission from the Journal of Clinical Pathology (1993 ; 46 : 198-203).

TABLE 1. ESR values (mm for verification of comparability of a working (routine) method with the ICSH standardized method. The values incorporate a correction for dilution of citrate in the working method. Proposed working method valid if 95% of results are within indicated limits.

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

[1]Standardized method - EDTA anticoagulated but undiluted whole blood of Hct 0.35 or less.
[2]Working method - 4 vol EDTA blood plus 1 vol citrate diluent.

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