Specification for

Westergren tubes and support for the measurement of erythrocyte sedimentation rate

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Committees responsible for this British Standard

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British Laboratory Ware Association
British Society for Haematology
Department of Health and Social Security
Institute of Medical Laboratory Sciences
Medical Research Council
Medical Sterile Products Association
Ministry of Agriculture, Fisheries and Food
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Society of Glass Technology

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Foreword

This revision of BS 2554 has been prepared under the direction of the Laboratory Apparatus Standards Committee. It was first published in 1954 and revised in 1968. This revision of BS 2554 supersedes the 1968 edition which is withdrawn.

The measurement of the erythrocyte sedimentation rate (ESR), that is the suspension stability of red blood cells, is a routine test in the investigation and management of certain diseases. As several factors may affect the rate, it is important to standardize the apparatus and procedure as far as possible. If it is not possible to obtain sufficient blood for this procedure, for example from babies, a micro-method may be adopted, with a 100 mm or 200 mm column of blood in a tube of much narrower bore; this method may give variable results when replicate measurements are made on aliquot portions of the same sample of blood, and it is particularly inaccurate when the packed cell volume is more than about 0.4 L/L. A third method, the Wintrobe method, was formerly used extensively in the USA and to some extent in Britain with the tube being the same as that used for estimating the packed red cell volume. The Wintrobe tube is now specified in BS 4316 for estimating packed red cell volume alone.

Since 1968 the development of new methods of establishing and maintaining a 200 mm column of blood has led to the introduction of disposable plastics and modified glass tubes for the measurement of the ESR by the Westergren method; these have largely replaced the tube specified in the 1968 edition of BS 2554. The present specification standardizes the features which are essential to the Westergren method, whether a single-use or re-usable tube is used. The re-usable tubes are the same as those specified in the 1968 edition.

The accuracy of results is greatly influenced by various technical factors to which it is essential to pay strict attention. Details are given in Appendix A of the recommended technique against which any modifications, found convenient for routine purposes, should be standardized.

The principal differences between BS 2554:1968 and this edition are:

- a) that requirements for single-use tubes are included;
- b) that requirements for supports are included;
- c) that subjective requirements for the material have been replaced by objective requirements.

CAUTION. This British Standard calls for the use of procedures which may be injurious to health if adequate precautions are not taken. Venepuncture, the setting-up of an ESR test, and the handling of a Westergren tube after completion of the test may all cause contamination to the operator with blood, and this may cause infection of the operator. This British Standard refers only to the technical suitability and does not absolve the user from legal obligations relating to health and safety at any stage.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, pages 1 to 10, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

Section 1. General

1 Scope

This British Standard specifies requirements for single-use and re-usable tubes for measuring the erythrocyte sedimentation rate (ESR) by the Westergren method, and for a support to hold tubes during the performance of the test.

A procedure for measuring the erythrocyte sedimentation rate by the Westergren method is given in Appendix A.

NOTE The titles of the publications referred to in this standard are listed on the inside back cover.

2 Marking

2.1 Graduation lines

- **2.1.1** Graduation lines shall be clean and of uniform thickness not greater than 0.3 mm.
- **2.1.2** Graduation lines shall lie in planes at right angles to the axis of each tube, and shall be without irregularity in their spacing.

2.2 Scale

- **2.2.1** A scale, graduated in millimetres, shall run downwards for at least 150 mm from a zero mark situated 200 mm above the lower end of each tube.
- **2.2.2** There shall be a distance of 1 mm between the centres of adjacent graduation lines.
- **2.2.3** The lengths of graduation lines shall be varied so as to distinguish clearly every tenth line and every intermediate fifth line as follows.
 - a) The length of the short lines shall be not less than 10 % and not more than 20 % of the circumference of the tube.
 - b) The length of the medium lines shall be approximately 1.5 times the length of the short lines. They shall extend symmetrically at each end beyond the ends of the short lines.
 - c) The length of the long lines shall be approximately twice the length of the short lines. They shall extend symmetrically at each end beyond the ends of the short and medium lines.

2.3 Figuring of graduation lines

- ${f 2.3.1}$ Every tenth (long) graduation line shall be figured.
- **2.3.2** Figures shall be at least 2 mm high and shall be placed immediately above the long line and not more than 1 mm to the right of the adjacent short graduation lines.

3 Inscriptions

The following inscriptions shall be marked on the tube and/or on the support:

- a) the symbol "mm" above "0" on the scale:
- b) the temperature "20 \pm 3 °C";
- c) the maker's and/or vendor's mark or name;
- d) the number and date of this British Standard, i.e. BS 2554:1987¹⁾.

The inscriptions shall be positioned so that they are visible to the operator when the tube is put in the support for which it is intended.

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¹⁾ Marking BS 2554:1987 on or in relation to a product is a claim by the manufacturer that the product has been manufactured to the requirements of the standard. The accuracy of such a claim is therefore solely the manufacturer's responsibility. Enquiries as to the availability of third party certification should be addressed to the appropriate certification body.

Section 2. Single-use Westergren tubes

4 Construction

4.1 Material

- **4.1.1** The tube shall be made from either rigid, transparent plastics or glass complying with class HGA 3 or better of BS 3473-3 so that:
 - a) the rigidity, when tested according to Appendix B, shall be such that the depression does not exceed 1 mm:
 - b) the transparency shall be sufficient to permit the top of the column of blood and the top of the red cell layer to be seen clearly in relation to the scale.
- **4.1.2** The tube shall be free from defects which impair observation of the top of the column of blood and of the top of the red cell layer.

NOTE The single-use Westergren tube should be reasonably free from internal stress.

4.1.3 The material of which the tube is made shall not affect the ESR value, when tested in accordance with the method described in Appendix C, by more than 6 mm.

4.2 General design

The general design of the single-use Westergren tube shall be as shown in Figure 1.

4.3 Straightness

The tube shall be straight when tested in accordance with the method described in Appendix D.

4.4 Finish

- **4.4.1** The glass tube shall be cut square (within 10°) with the axis of the tube, and shall be lightly fire polished at each end.
- **4.4.2** The plastics tube shall be cut square (within 10°) with the axis of the tube. The ends shall be smooth and may be slightly bevelled.
- **4.4.3** The tube shall be supplied free from any contamination which would affect the ESR value when tested in accordance with the method described in Appendix C.
- **4.4.4** The tube, by itself or in association with its support, shall have a mechanism which ensures that the tube remains filled with blood, from its lower end to the zero mark on the scale, during the 60 min required to determine the ESR.

4.5 Dimensions

The measuring part of the tube shall comply with the dimensions given in Table 1.

Table 1 — Essential dimensions of a single-use Westergren tube

0				
	mm			
Internal diameter	2.55 ± 0.15			
Ovality of bore	less than 0.1			
Length of measuring part	200 ± 1			
NOTE Ovality of bore is the difference between maximal and				

NOTE Ovality of bore is the difference between maximal and minimal diameters at any cross section of the tube.

5 Graduation and figuring

Graduation or figuring shall comply with clause 2.

6 Inscriptions

Inscriptions shall comply with clause 3.

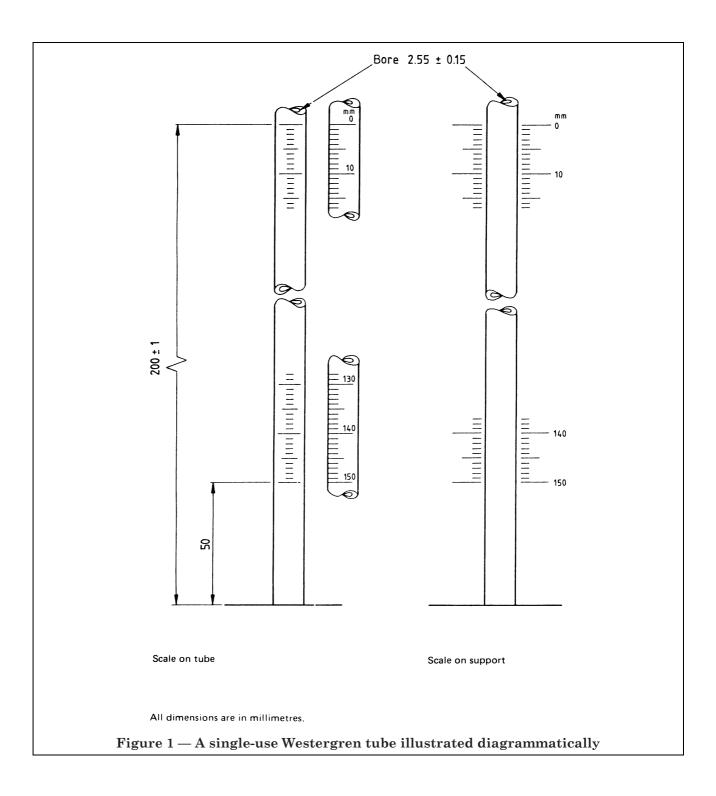
 $\ensuremath{\mathsf{NOTE}}$ Additionally the inscription "single-use Westergren ESR tube" may be marked.

7 Labelling

Each package of single-use Westergren tubes shall be clearly labelled with at least the following information:

- a) the words "Single-use Westergren ESR tubes";
- b) the words "Ready for use";
- c) the temperature "20 \pm 3 °C";
- d) the makers' and/or vendor's name or mark;
- e) an identifying reference to the batch of manufacture;
- f) the number and date of this British Standard, i.e. BS 2554:1987²⁾.

²⁾ Marking BS 2554:1987 on or in relation to a product is a claim by the manufacturer that the product has been manufactured to the requirements of the standard. The accuracy of such a claim is therefore solely the manufacturer's responsibility. Enquiries as to the availability of third party certification should be addressed to the appropriate certification body.



Section 3. Re-usable Westergren tubes

8 Construction

8.1 Material

8.1.1 The tube shall be made from transparent, thick-walled glass tubing complying with class HGB 3 or better of BS 3473-2.

NOTE The tube should be as free as possible from visible defects and reasonably free from internal stress.

8.1.2 The tube shall be free from defects which impair observation of the top of the column of blood and the top of the red cell layer.

8.2 General design

The general design of a re-usable Westergren tube shall be as shown in Figure 2.

8.3 Straightness

The tube shall be straight when tested in accordance with the method described in Appendix D.

8.4 Finish

- **8.4.1** The upper end of the tube shall be ground smooth and square with the axis of the tube, and shall be slightly bevelled.
- **8.4.2** The lower end of the tube shall be tapered as shown in Figure 2, and the tapered portion shall be finely ground or polished.
- **8.4.3** The specified bore of the tube shall be maintained throughout, and shall not be drawn down to form the jet.

8.5 Dimensions

The tube shall comply with the dimensions given in Table 2.

Table 2 — Dimensions of a re-usable Westergren tube

Westergreif table					
	mm				
Overall length	300 ± 1				
External diameter	6.5 ± 0.5				
Internal diameter (bore)	2.55 ± 0.15				
Ovality of bore	less than 0.1				
Length of measuring part	200 ± 1				
Length of tapering portion	6 ± 2				
Wall thickness of orifice	at least 0.5				
NOTE O 1: 61 : 11 1:66 1 1 1					

 ${
m NOTE}$ Ovality of bore is the difference between maximal and minimal diameters at any cross section of the tube.

9 Graduation and figuring

- 9.1 Markings shall be clean and permanent.
- **9.2** Graduation lines, scale and figuring shall comply with clause **2**.

10 Inscriptions

Inscriptions shall comply with clause 3.

 $\ensuremath{\text{NOTE}}$ $\ensuremath{\text{Additionally}}$ the inscription "re-usable Westergren ESR tube" may be marked.

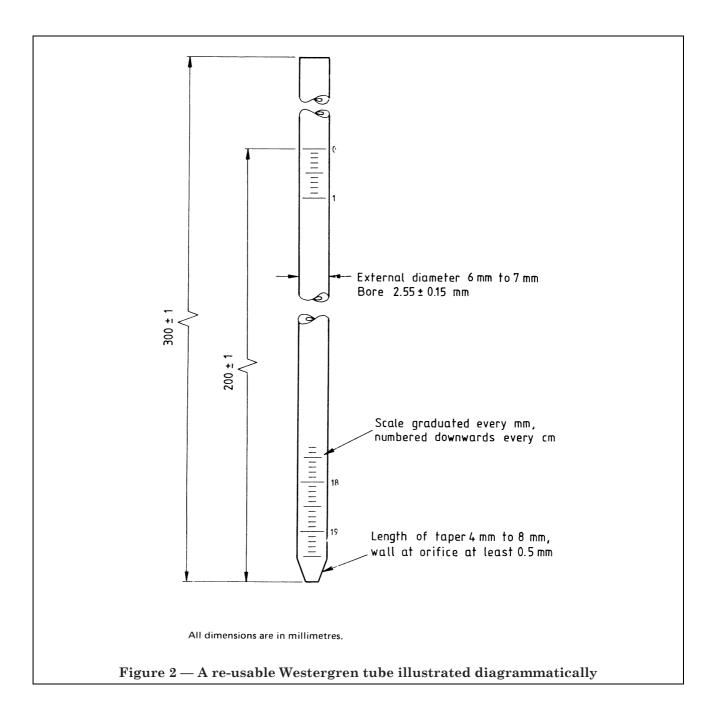
11 Labelling

Each package of re-usable Westergren tubes shall be clearly labelled with at least the following information:

- a) the words "Re-usable Westergren ESR tubes":
- b) the temperature "20 \pm 3 °C";
- c) the words "Wash before use in acetone/water";
- d) the words "Disinfect after use";
- e) the maker's and/or vendor's name or mark;
- f) an identifying reference to the batch of manufacture;
- g) the number and date of this British Standard, i.e. BS $2554:1987^{3}$.

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³⁾ Marking BS 2554:1987 on or in relation to a product is a claim by the manufacturer that the product has been manufactured to the requirements of the standard. The accuracy of such a claim is therefore solely the manufacturer's responsibility. Enquiries as to the availability of third party certification should be addressed to the appropriate certification body.



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Section 4. Support for Westergren tubes

12 Construction

12.1 The support shall be a rigid structure having clips or holes to hold rigidly one or several Westergren tubes, and shall be fitted with either a plumb-line or spirit-level. The support shall stand on three feet, two of which shall be adjustable.

NOTE The adjustable feet and the plumb-line or spirit-level are provided to permit adjustment to ensure that the tubes are held within 1° of the vertical.

12.2 When erythrocyte sedimentation rates are to be measured against scales marked on the support, the scales shall be marked on a surface fixed vertically behind the tubes and not more than 4 mm from each tube.

12.3 The support shall be constructed of such materials, and in such a way, that it is able to withstand repeated disinfection in the laboratory.

13 Graduation and figuring

13.1 Scales, figuring and inscriptions shall be provided on the support, if not marked on the tubes, and shall comply with clause **2**.

NOTE A re-usable tube is intended for use with an appropriate support forming a system. The distribution of markings between tube and support may differ between one system and another.

13.2 Markings on the support shall be permanent.

13.3 Scales

When scales are provided on a support they shall be fixed behind every tube, within 4 mm of the tube, as shown in Figure 1.

13.4 Figuring

Every figure shall be at least 2 mm high, and shall be placed not more than 1 mm from the right-hand end of the graduation line to which it refers in such a way that an extension of the line would bisect the figure.

14 Inscriptions

- 14.1 Inscriptions shall comply with clause 3.
- **14.2** The following additional inscriptions shall be marked:
 - a) the recommended method for the disinfection of the support after use;
 - b) the inscription "Westergren ESR".

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Appendix A Measurement of the erythrocyte sedimentation rate by the Westergren method

A.1 Principle

A 200 mm column of venous blood, diluted with trisodium citrate solution, is allowed to stand undisturbed for 60 min at 20 ± 3 °C and the height of the sediment determined.

NOTE No correction is made for anaemia.

A.2 Reagents

A.2.1 Trisodium citrate solution ($Na_3C_6H_5O_7$), 0.11 ± 0.01 mol/L. Dissolve either 32.8 g of $Na_3C_6H_5O_7.2H_2O$ or 39 g of $Na_3C_6H_5O_7.5\frac{1}{2}H_2O$ in 1 L of distilled water. Filter through a sterile membrane of maximal pore diameter 0.22 μ m in to a sterile container and store at 4 °C. Before use examine visually for freedom from particles and moulds.

A.3 Apparatus

A.3.1 *Support*, which shall hold the tube rigidly and to within 1° of the vertical, and shall keep the tube filled with blood to the zero mark.

A.4 Conditions of test

The test shall not be performed in direct sunlight, near a heat source or in a draught.

The tube shall not be subjected to vibration during the test.

The test shall be performed in a stable environment at a temperature of 20 ± 3 °C.

NOTE 1 The performance of the test at a temperature outside this range, and variation in the temperature during the test, may cause considerable variation in results.

NOTE 2 If tests are performed at a temperature other than 20 ± 3 °C the corresponding normal range of erythrocyte sedimentation rate should be determined.

A.5 Preparation of venous blood

Add 4 volumes of venous blood, obtained by clean venepuncture, to 1 volume of sodium tricitrate solution (**A.2.1**) and mix well. The dilution error shall not exceed 5 %.

NOTE The venous blood may be added directly to the sodium tricitrate solution or may first be anticoagulated with 1.3 \pm 0.1 g of anhydrous ethylenediaminetetra-acetic acid (EDTA) per mL of blood, and then, within 2 h, added to the citrate solution or to a 9 g/L sodium chloride solution.

A.6 Procedure

WARNING. It is essential that mouth pipetting is not used because of the danger of infection, and that filling is done mechanically.

A.6.1 Start the measurement of the erythrocyte sedimentation within 2 h of venepuncture.

A.6.2 Fill the Westergren tube from the lower end to the zero mark with the diluted blood (prepared as in **A.5**) which has been well mixed immediately before use.

A.6.3 Place the tube filled with the diluted blood solution in the support (**A.3.1**).

A.6.4 Allow the tube to remain undisturbed for 60 ± 1 min and then immediately read from the scale the upper limit of the red cell layer.

NOTE After use, re-usable tubes should be disinfected, then cleaned in acetone/water and dried. Detergent or dichromate should not be used for cleaning the tubes.

A.7 Expression of results

The sedimentation result is expressed as millimetres in the first hour (Westergren).

A.8 Interpretation of result

The result is compared with the values given in Table $3^{4)}$.

Table 3 — Erythrocyte sedimentation rate (Westergren) in Britain (mm/1st h)

Age	Mean normal	Significantly abnormal when above
years	mm/1st h	mm/1st h
Men:		
17 to 50	4	10
51 to 60	6	12
over 60	6	14
Women:		
17 to 50	6	12
51 to 60	9	19
over 60	10	20
Children:		
< 17	4	10

Appendix B Test for rigidity of a Westergren tube

B.1 Principle

The depression (in mm) of a Westergren tube supported at the 10 mm and 150 mm marks when a load of 100 g is applied at the 80 mm point is determined.

B.2 Apparatus

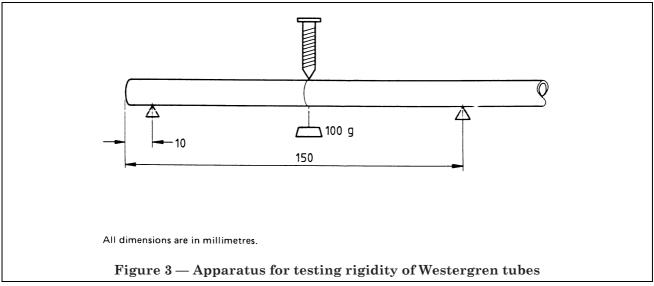
B.2.1 A vertically working screw micrometer.

B.3 Procedure

B.3.1 Set up the apparatus as illustrated in Figure 3.

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⁴⁾ Values taken from Dacie, J.V. & Lewis, S.M. *Practical haematology*, 6th ed., London: Churchill, 1984.



B.3.2 Support a tube horizontally on knife-edges placed under the 10 mm and 150 mm points.

B.3.3 Mark the position of the upper surface of the tube at the 80 mm point using the vertically working screw micrometer (**B.2.1**).

B.3.4 Suspend a load of 100 g at the 80 mm point and measure the depression of the tube at this point with the micrometer. Record this value.

B.4 Expression of results

Express the depression in millimetres.

Appendix C Test for contamination and interfering substances

C.1 Principle

The difference in mean ESRs between a set of three tubes under test and three re-usable tubes is determined by using aliquot portions of the same blood and under the same conditions.

C.2 Apparatus

C.2.1 Thoroughly cleaned re-usable Westergren tubes (see note to A.6.4).

C.3 Sample of tubes under test

A set of three single-use Westergren tubes, selected at random from a batch representative of the type under test.

C.4 Preparation of blood sample

Prepare 15 mL of diluted blood according to **A.5**, using blood of one group and an ESR between 50 mm/1st h and 120 mm/1st h.

C.5 Procedure

Perform the procedure described in **A.6**, but fill the three tubes under test selected as described in **C.3** and three re-usable tubes (**C.2.1**) with the diluted blood prepared as described in **C.4**.

C.6 Expression of results

Calculate the mean ESR value for each of the set of three tubes. Calculate the difference between the two mean results and report this as the result of the test.

Appendix D Test for straightness of a Westergren tube

D.1 Principle

The deviation from straightness of the tubes is assessed, using a straightness device.

D.2 Apparatus

D.2.1 Straightness device, having two knife-edges 140 mm apart with a third knife-edge midway between the other two and recessed 1 mm behind the line joining them, as shown in Figure 4.

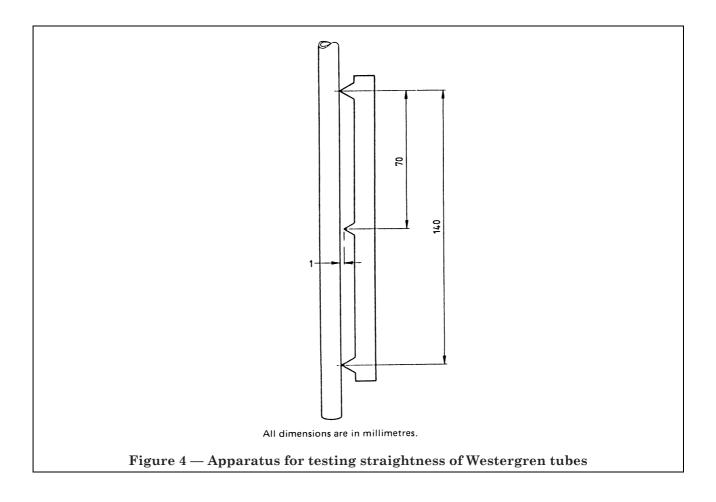
D.3 Procedure

Support the tube under test in the vertical position. Apply the straightness device (**D.2.1**) to the side of the tube, as shown in Figure 4, and rotate the tube through 360°. Observe whether the middle knife edge comes in contact with the surface of the tube.

D.4 Expression of results

If the middle knife-edge is not observed to make contact with the surface of the tube then the tube shall be deemed to be "straight".

If the middle knife-edge is observed to make contact with the surface of the tube then the tube shall be deemed to be "not straight".



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Publications referred to

BS 3473, Chemical resistance of glass used in the production of laboratory glassware. BS 3473-2, Method for determination of hydrolytic resistance of glass grains at 98 °C. BS 3473-3, Method for determination of hydrolytic resistance of glass grains at 121 °C. BS 4316, Specification for apparatus for measurement of packed red cell volume⁵⁾. Dacie, J.V. & Lewis, S.M. Practical haematology, 6th ed., London: Churchill, 1984.

 $^{^{5)}}$ Referred to in the foreword only.

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