

For qualitative estimation of the Erythrocyte Sedimentation Rate (ESR or Sed-Rate) on vacuum blood collection tubes.

For In Vitro Diagnostic Use Only

Contents: (sufficient for 100 ESR determinations)
100 Vacupettes* with pistons.

***Note:** The word Vacupette or Vacupettes used throughout this document is the Trade Name for the Disposable ESR pipette with piston manufactured by Guest Scientific AG.

Storage Conditions:

Vacupette may be stored at room temperature, which may be cold, cool or warm: However the test itself must be performed at temperatures between 18 - 25°C (64 - 77°F) see note c) page 3.

Background: The erythrocyte sedimentation rate (ESR) is one of the most widely requested laboratory tests throughout the world and vacuum blood collection systems are increasing in popularity with Clinicians and Phlebotomists. Vacupette bridges the gap between the traditional Westergren tube dimensions and the modern vacuum blood collection tubes.

While infection may be the most common cause of elevated ESR results, many other conditions such as malignant tumours and renal disease have been associated with raised values; hence its role has become established in the Clinician's mind as a screening test for the presence of clinical illness.^{1,2,3}

Elevated ESR results correlate well to the severity of acute inflammatory disease and the test has been cited as a useful indicator of stroke severity and predictor of early relapse or survival in Hodgkin Disease.^{4,5,6}

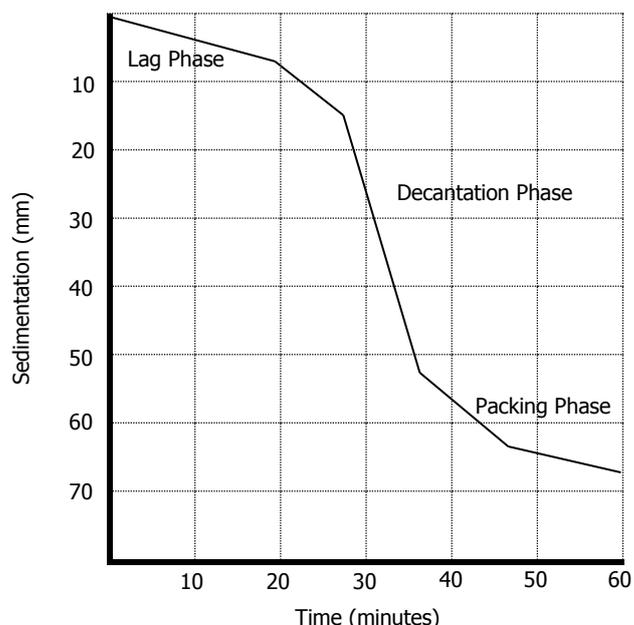
In 1926 Alf Westergren, published 'The Technique of the Red Cell Sedimentation Reaction',⁷ based on the work of Robin Fahreus in 1918. His technique included the use of a combined diluent and anticoagulant (tri-sodium citrate) for the blood sample and specified the length (200mm) and bore diameter (2.5mm) of the measuring tube. His technique became the basis of the test we know today as the Westergren method and the pipet dimensions and dilution factor were adopted as the reference method for erythrocyte sedimentation rate by the International Committee for Standardization in Haematology (ICSH) in 1973.⁸

Since 1926 various workers have proposed variations to the Westergren method, (e.g. Wintrobe in 1935 and the 'zeta' sedimentation rate in 1972), however in 1993 the ICSH stated that a slightly modified Westergren method should be used for the reference method to which all other techniques, if not conforming, should be standardised.⁹ The most recent review by the ICSH published in April 2011 confirms that the reference method for measurement of the ESR should be based on the Westergren method using diluted blood.¹⁰ In similar manner the most recent CLSI Approved Standard (5th Ed.), for ESR recognises the Westergren method as the standardized or selected procedure.¹¹

The Vacupette pipette conforms to Westergren dimensions.

Physical basis of blood sedimentation: To this day the phenomenon of erythrocyte sedimentation is still only partly understood, however three definite phases of the process have been identified: During the first, or Lag Phase, the red cells form a characteristic rouleaux pattern and sedimentation is generally slow. The rate accelerates in the second period, the Decantation Phase and slows again in the final Packing Phase as the red cell aggregates pile up towards the lower part of the tube.

Fig1. The Phases of Blood Sedimentation.



Note: Sedimentation is not linear and the time taken over each of the Lag, Decantation and Packing phases will differ between patients, hence the observer must never try to 'estimate' the final result before the full 60 minute time period for the test has elapsed. The size of rouleaux aggregates formed in the Lag Phase is the critical factor affecting the final result. Rouleaux appears to be mainly influenced by plasma proteins including fibrinogen, IgM and alpha₂-macroglobulin. Opinions vary as to the accelerating and retarding properties of glycoprotein and albumin. IgG appears to increase the sedimentation rate only at high concentrations.

Items required but not provided in the Vacupette box:

1. An ESR stand to hold the Vacupette and test tube assemblies in a vertical position, controlled by means of a levelling bubble and adjusting screws (code: GS or FH 1705).
2. A laboratory or similar timer capable of alarming after exactly one hour (60 minutes) elapsed time.

Sample requirement:

Vacupette is designed to fit into the standard size vacuum extraction tubes, pre-citrated for ESR (black stopper), that contain 3.3%, 0.109mol/L tri-sodium citrate; C₆H₅O₇Na₃ - 2H₂O; CAS no. 6132-04-3 giving a dilution ratio of 1 part citrate to 4 parts blood.

Note: Blood samples anticoagulated with Sodium citrate for coagulation studies are not suitable for ESR testing.

Whole blood should be obtained by clean venipuncture over a maximum period of 30 seconds without excessive stasis.

Once taken the blood sample and anticoagulant mixture must be immediately mixed gently, but thoroughly at least 8 times by complete inversion of the sample tube.

The most recent ESR review¹⁰ states that citrated blood samples must be tested within 2 hours if left at room temperature, or 4 hours if stored at 4°C.

Note: In the USA the most recent CLSI guidance¹¹ states that the blood sample may be stored for up to 24 hours in a refrigerator.

Blood specimens stored in a refrigerator must return to room temperature naturally before testing starts.

If a sample is left for any amount of time before testing, either at room or refrigerator temperature, it must be well mixed by at least 8 gentle inversions of the tube before the test is started. (see paragraph 1 under Instructions for use.)

Instructions for Use:

Note: Always wear appropriate personal protective clothing as indicated by Good Laboratory Practice. If blood is spilled follow your laboratory policy or national guidance for safe disposal.

1. Mix the blood sample tube thoroughly but gently, by at least 8 full inversions.
2. Remove the black stopper from the vacuum tube & carefully insert the vacupette, blue piston end first, into the mouth of the tube (diagram 2).
3. Gently push the Vacupette further into the tube and observe the blood sample being pushed up inside the bore until it reaches just beyond the zero level (diagram 3). The blood sample must be seen to be slightly absorbed by the cotton plug. Keep the Vacupette and piston at that level. Do Not push the pipette further in or try to pull it out.
4. Place the full Vacupette and vacuum tube assembly in a correctly levelled ESR stand ensuring that the Vacupette remains vertical, i.e. at 90 degrees, plus or minus one degree to the horizontal, (diagram 4).
Immediately start the timer.
5. At the end of the timed hour, the result is read by aligning the eye to the level where the red cell column has dropped – leaving clear plasma above (see illustration d on page 3). Record the number of mm the red cells have dropped from the mm scale printed on the Dispette.
6. Record the result as ESR = x mm
or Sed-Rate = x mm

Note: It is common, though an incorrect practice, to record ESR results as x mm/hour or x mm per hour. Both the ICSH and CLSI point out that despite the common title Erythrocyte Sedimentation *Rate*, it is not in fact a rate but a measurement at one hour. Hence both these official organizations currently recommend that results should be reported as: ESR = x mm.

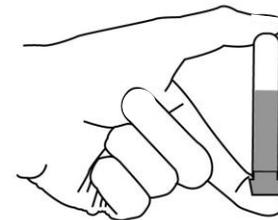
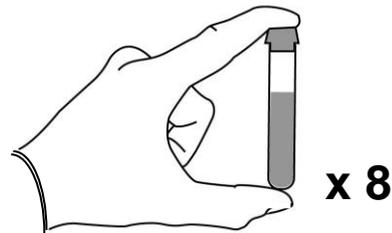


Diagram 1.

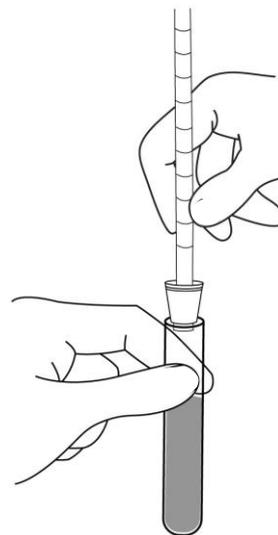


Diagram 2.

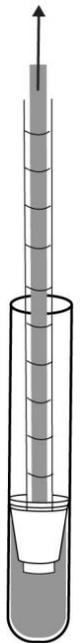


Diagram 3.

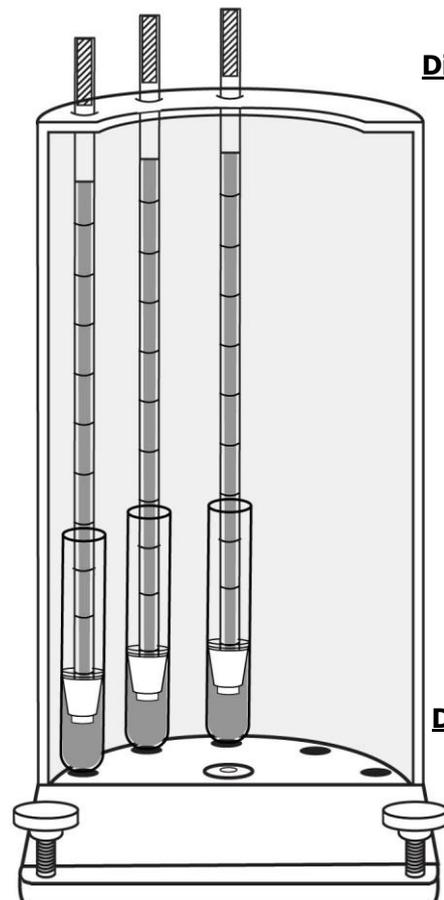


Diagram 4.

PRECAUTIONS:

- a) Thorough mixing of the blood sample is essential see 1. under instructions for use. However it must be done gently, shaking the sample will result in haemolysis which may obscure the end point.
- b) Vigorous mixing may also create bubbles which will seriously affect the result. If bubbles are present in the Vacupette repeat the test with a fresh sample that has been more carefully mixed.
- c) The ESR must be performed at room temperature (defined by the ICSH as 18 to 25°C or 64 to 77°F). Do not place the stand near a window, in direct sunlight or where it may be subjected to drafts.
Note: Should it be impossible to perform the test within this temperature range request a temperature correction chart from Arkray USA Inc.
- d) The ESR is affected by vibration; ensure the stand is isolated from machinery and that the bench is not subjected to knocks.
- e) Do not pick up the stand to read the result as this will affect other tests in progress. Bring the eye to the level of the red cells to read accurately from the scale (see illustration d).
- f) Be careful to ensure that the Vacupette pipette remains upright in the ESR stand for the one hour duration of the test. If knocked, the vacuum tube and Vacupette may bend at the piston point thus slewing the Vacupette away from the vertical.
- g) Occasionally the level of the red cells is not clear-cut and a 'Christmas Tree' effect may be observed. In such cases the level where the red cells become fully concentrated should be recorded (see illustration e).
- h) In cases of serious infection or leukaemia a heavy layer of white cells may be present on top of the column of red cells. This should be ignored and the reading taken from the level of the red cells only.
- i) It must be remembered that the red cells will continue to settle after one hour has elapsed. Therefore it is most important to read the test at exactly one hour after setting up the test in the stand (e.g. the test reading will generally be higher at 1 hour 15 minutes than at 1 hour).
- j) It must not be assumed that the reading at 60 minutes will be twice that at 30 minutes because the rate of fall is not linear (see Physical basis of blood sedimentation on page 1)

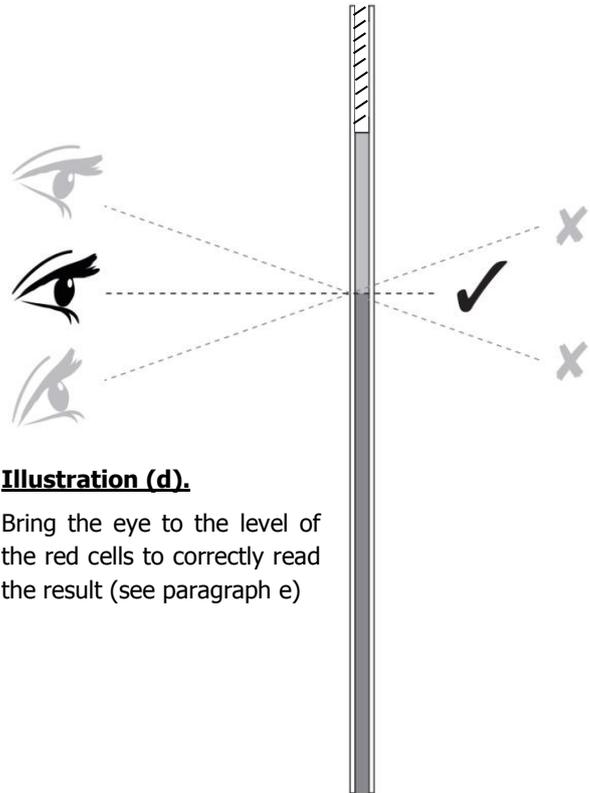


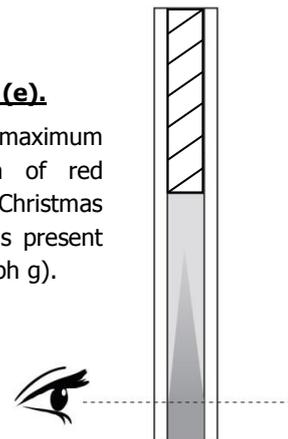
Illustration (d).

Bring the eye to the level of the red cells to correctly read the result (see paragraph e)

Please note: Illustrations are not to scale

Illustration (e).

Reading the maximum concentration of red cells where 'Christmas Tree' effect is present (see paragraph g).



Calibration, Quality Control and Normal Values:

For calibration we recommend reference to the ICSH Review of Measurement of erythrocyte sedimentation rate, published in April 2011,¹⁰ the original ICSH paper published in 1993,⁹ or the U.S. equivalent: CLSI Approved Standard H02- A5.¹¹

Some commercial companies offer quality control samples for ESR; however we have not been able to compare them to the ICSH reference method. It is recommended that laboratories must therefore instigate and record their own quality control system in accordance with ICSH or CLSI guidance and/or their own country's regulatory requirements.

As normal values for the ESR can vary with age and ethnic origin, reference to the ICSH or CLSI document should also be made for guidance on normal values appropriate to individual countries.

For a list of Frequently Asked Questions about ESRs please go to www.guestscientific.com & follow the links from the drop down menu.

Text References

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11. Clinical and Laboratory Standards Institute (CLSI), Procedures for the Erythrocyte Sedimentation Rate Test; Approved Standard-Fifth Edition. H02-A5, May 2011. ISBN 1-56238-754-5.

Vacupette is manufactured in Switzerland by:

Guest Scientific AG

Gewerbestrasse 10

CH-6330 Cham



++41 (0)41 741 4748

Fax ++41 (0)41 741 3966

email: guest.scientific@bluewin.ch

web: www.guestscientific.com

Distributor: