**Dispette 2 saline**

*For qualitative estimation of the Erythrocyte Sedimentation Rate (ESR or Sed-Rate)*

*For In Vitro Diagnostic Use Only*

**Contents:** (sufficient for 100 ESR determinations)

100 plugged Dispette 2 pipets (Westergren dimensions) plus 100 filling reservoirs containing 0.25ml Normal saline diluent. This solution is not defined as a hazardous material under the OSHA Hazard Communication standard (29 CFR 1910.1200).

**Storage Conditions:**

Reservoirs should be stored in a cool, dry place at 50 to 75°F (10 to 24°C). Never freeze the Dispette 2 reservoir.

Evaporation of saline in the reservoir can affect the ESR result. To know if your product has evaporated beyond acceptable levels please observe when the saline in the reservoir **FAILS** the following checklist:

Check the saline in the reservoir BEFORE EVERY USE to ensure:

- All saline is at the BOTTOM of the reservoir
- Check saline solution is CLEAR.
- Check saline volume is NOT MORE THAN 1 mm below the saline check level (see illustration)

If the saline is MORE THAN 1 mm BELOW the saline check level, or if it is cloudy, the reservoir MUST be discarded.

**Background:** The erythrocyte sedimentation rate (also known as the ESR or Sed-Rate) is one of the most widely requested laboratory tests and manual methods, such as the Dispette 2, are particularly popular in the outpatient's setting in the USA.1

While infection may be the most common cause of elevated results, many other conditions such as malignant tumours and renal disease have been associated with raised values; hence its role in the Clinician's mind as a screening test for the presence of clinical illness has become established.2,3,4

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 and survival in Hodgkin Disease.5,6,7

The work of Robin Fahreus in 1918 defined almost all the important characteristics of the test as it is performed today. In 1926 Alf Westergren, published 'The Technique of the Red Cell Sedimentation Reaction', 8 which 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 Westergen method and the dimensions and dilution factor were adopted as the reference method for erythrocyte sedimentation rate by the International Committee for Standardization in Hematology (ICSH) in 1973.8

Over time several workers have proposed variations to the Westergen method, those of some note being the Wintrobe technique (1935) and Bull & Brailsford's 'zeta' sedimentation rate (1972), however in 1993 the ICSH stated that the Westergen method and dimensions should be used for the reference method to which all other techniques, if not conforming, should be standardised.9,10

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 Westergen method using diluted blood.11 In similar manner the most recent CLSI Approved Standard (5th Ed.), for ESR recognises the Westergen method as the standardized or selected procedure.12

The Dispette 2 system conforms to the dimensions and dilution factor of the Westergen method, but being a closed system, and disposable, offers operators a greater margin of protection from infection by blood borne viruses.

**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**

Please note that the sedimentation 'rate' is not linear and remember the time taken over the Lag, Decantation and Packing phases will differ between patients, hence the observer must never try to 'estimate' or 'guess' 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. The rouleaux appears to be mainly influenced by certain plasma proteins including fibrinogen, IgM and alpha-1-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 Dispette2 box:**

1. Transfer pipets.
2. A Guest ESR stand to hold the Dispette2 pipet and reservoir assemblies in a vertical position, controlled by means of a leveling bubble and adjusting screws. (code: GS or FH 1705)
3. A laboratory or similar timer capable of alarming after exactly one hour (60 minutes) elapsed time.

**Sample requirement:**

Whole blood should be obtained by clean venepuncture over a maximum period of 30 seconds without excessive stasis. A traditional syringe or a vacuum extraction system may be used. The sample should be taken into an EDTA anticoagulant tube and immediately well mixed by 8 gentle inversions of the container. The most recent ICSH review 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 current CLSI guidance 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 the 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 transfer to the reservoir.
Instructions for Use:

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

1. Select a translucent blue filling reservoir from the box and perform the following check:
   - Ensure all saline is at the BOTTOM of the reservoir
   - Check saline solution is CLEAR.
   - Check saline volume is NOT MORE THAN 1 mm below the saline check level (see diagram 1)

   If the volume of saline is MORE THAN 1 mm BELOW the saline check level or if it is cloudy, the reservoir MUST be discarded.

   Having established the correct volume of saline, keep reservoir upright and carefully remove cap.

2. Using a transfer pipet add **1ml well mixed EDTA anticoagulated whole blood** to the reservoir. Total volume of saline + blood should come up to the blood fill level (see diagram 2).

3. Ensure that the blood/saline mixture reaches, but does not go beyond the Blood fill level (see diagram 3) before replacing the cap securely.

4. Gently mix the reservoir, either mechanically or manually by inversion. A minimum of 8 inversions are recommended.

5. Before proceeding ensure that all the blood/saline mixture returns to the bottom section of the reservoir (diagram 5).

6. While firmly holding the filling reservoir with one hand and the Dispette2 pipet with the other hand positioned at the 180mm mark, penetrate the cap membrane and stop.

7. Gently continue inserting the Dispette2 pipet to the bottom of the reservoir causing the blood/saline mixture to rise up the inner bore of the pipet until it reaches to or beyond the blue grommet at the zero level (diagram 8a). The Dispette2 pipet must be fully inserted to the bottom of the reservoir – any excess of blood will be accommodated by the plugged overflow chamber.

8. Place the full Dispette2 assembly (pipet and reservoir) in a correctly levelled ESR stand ensuring that the pipet is at 90 degrees, plus or minus one degree, to the horizontal (diagram 8b). Immediately start the timer.

9. 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). Record the number of mm the red cells have dropped from the mm scale printed on the Dispette2 pipet.

10. Record the result as ESR = x mm
    (note: in some countries the term Sed-Rate = x mm would be acceptable, also see footnote on page 3)
PRECAUTIONS:

a) Thorough mixing of the blood sample both before adding to the reservoir and immediately before inserting the Dispette2 pipet into the reservoir is essential. However it must be done gently, shaking the sample will result in haemolysis which may obscure the end point.

b) Shaking the sample also creates bubbles which will seriously affect the result. If bubbles are present in the pipet repeat the test with a fresh ESR test preparation 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.

d) The ESR is affected by vibration; ensure the stand is placed well away from machinery and that the bench is not subjected to knocks. Remember that vibration may only occur intermittently and/or may be as a result of machinery (e.g. a centrifuge) sited in another part of the building.

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) If the result falls between 130 and 155mm, the exact end point may be obscured by the filling reservoir cap. In this case report the result as >130 mm. If a numerical value is requested estimations can be made (but are not recommended), by reading from the scale of an unused Dispette2 pipet held next to the test Dispette2 assembly.

   **Note:** Before reporting an estimated result be sure to verify that this complies with your laboratory’s best practice and standards. Record and report that the result has been estimated.

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 f).

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 (f).

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

Footnote relating to paragraph 10 of Instructions for Use:

It is common, though 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 title Erythrocyte Sedimentation Rate, it is not in fact a rate but a measurement at one hour. Hence both organisations currently recommend that results should be reported as: ESR = \( x \) mm.
Calibration, Quality Control and Normal Values:

For calibration we recommend the ICSH reference method detailed in the ICSH recommendations for measurement of erythrocyte sedimentation rate,10 or the U.S. equivalent: CLSI Approved Standard H02-A5.12

Some commercial companies offer quality control samples for ESR; however we have not been able to compare them to the ICSH reference method on which the Dispette2 system is based – Guest Scientific recommends 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 documents 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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