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Erythrocyte Sedimentation Rate Modified Westergren Method-RO

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This procedure guides the tech through performing an erythrocyte sedimentation rate using the modified westergren method.

II. PRINCIPLE:

A. The erythrocyte sedimentation rate is the rate in millimeters per hour that erythrocytes settle out of the plasma in a column of anticoagulated whole blood. The rate of red blood cell settling is affected by three factors: (1) red blood cell number and shape; (2) mechanical and technical factors (e.g. temperature, tube diameter, bubbles, etc.); and (3) plasma factors. When the first two factors are constant, the plasma composition of the blood becomes the single most important factor. The presence of fibrinogen, alpha-2-globulin, beta and gamma globulins decrease the red blood cell zeta potential promoting rouleaux which results in a faster sedimentation rate. The erythrocyte sedimentation rate thus reflects changes in plasma proteins which accompanies acute and chronic infections, inflammation, malignancy, multiple myeloma, macroglobulinemia and hyperfibrinoginemia. The major disadvantage of this procedure is that the sedimentation rate is dependent on RBC number and is prolonged by anemia. The erythrocyte sedimentation rate is used primarily to follow the progress of inflammatory diseases such as rheumatoid arthritis during treatment.

B. The modified Westergren method produces the same results as the Westergren erythrocyte sedimentation rate, but employs EDTA anticoagulated blood rather than citrate. The EDTA blood is diluted 4:5 with saline because undiluted EDTA yields results with poor precision.

III. SPECIMEN COLLECTION AND HANDLING:

Type	Whole blood collected in a 4 mL vacutainer **Microtainer specimens are NOT acceptable!**	
Anticoagulant	K_2EDTA	
Amount	Whole blood	Minimum sample size is 2.0 mL Optimum sample size is 4.0 mL
Special Handling	Specimen must be well mixed for minimum of two minutes before being analyzed.	
Timing	Samples containing sickle cells, spherocytes, lipemia, or cold agglutinins may	

	give false results. Specimen is stable for 4 hours room temperature (20-25^{o}C) and 24 hours refrigerated (2-8^{o}C).
Criteria for Unac-ceptable Specimens	Specimens containing clots or inappropriate volumes are unacceptable and must be redrawn.

IV. SUPPLIES:

A. Fisherbrand™ Dispette™ 2 Disposable ESR kit. This includes:
   1. Triple-plugged Dispette™ pipettes, calibrated in 200mm
   2. Filling reservoirs with cap containing 0.25mL of 0.9% Saline
      a. Store at 10-24^{o}C. Keep away from sunlight. Never freeze reservoirs.

B. Westergren pipette rack

V. QUALITY CONTROL (QC):

The Dispette™ method is only a backup for our two automated instruments and is utilized only when both automated instruments are down or if *i*SED verification is needed. If that should occur, the commercial control utilized for the automated instruments can be run by the Dispette™ method. Results should be documented on the designated logsheet. Alternatively, a whole blood specimen with a hematocrit of 35 ± 1 that has been run on either of the *i*SEDs may be used as a control. Document results on the Dispette QC log. (See Attachment.)

VI. PROCEDURE:

A. Verify the Westergren pipette rack is correctly leveled, adjusting the legs until the bubble is centered.

B. Select a filling reservoir; shake all liquid to the bottom. Avoid bubbles. If the liquid is more than 1mm below the diluent line or the liquid is not clear, the filling reservoir must be discarded. (See Figure 1.)

C. Keep the filling reservoir upright and remove the cap.

D. Add 1 mL well mixed EDTA whole blood to the reservoir. After dispensing, the blood should be at the filling line. (See Figure 1.) Replace the cap.

E. After replacing the cap, **carefully** mix the filling reservoir mechanically or manually a minimum of 12 complete inversions with the air bubble traveling from end to end. **Do not shake the reservoir. Avoid bubbles.**

F. Hold the reservoir upright. Wait until all liquid has returned to the bottom and check for bubbles.

G. While firmly holding the filling reservoir with one hand and the Dispette™ pipette tube with the other hand positioned at the 180 mm mark, gently insert the pipette tube through the cap membrane and stop. (See Figure 2.)

H. Gently continue inserting the Dispette™ tube to the bottom of the reservoir. (See Figure 3.) Ensure that the blood level, rising up the Dispette™ tube, reaches to or beyond the grommet at the zero level (Figure 4).**The pipette must be fully inserted to the bottom.** When the Dispette™ tube is fully inserted, any extra blood will be accommodated by the plugged overflow chamber. The cotton piece may move within the two blue grommets. The lower blue grommet will ensure the correct level of blood in the column. **The column of blood must not have any air bubbles.**

I. Place full Dispette™ pipette assembly in the Westergren rack making sure the tube stands vertical. Make sure the rack is still level. See Notes 1, 2, 3, and 4.

J. Set timer for 60 minutes.

K. In exactly 60 minutes, read level of RBC fall in millimeters. The result is read by aligning the eye to the upper end of the red cell column.

1. **NOTE:** If the demarcation between the plasma and red cell column is hazy, the level is taken where the full density is first apparent.
2. Images: Figures 1-4

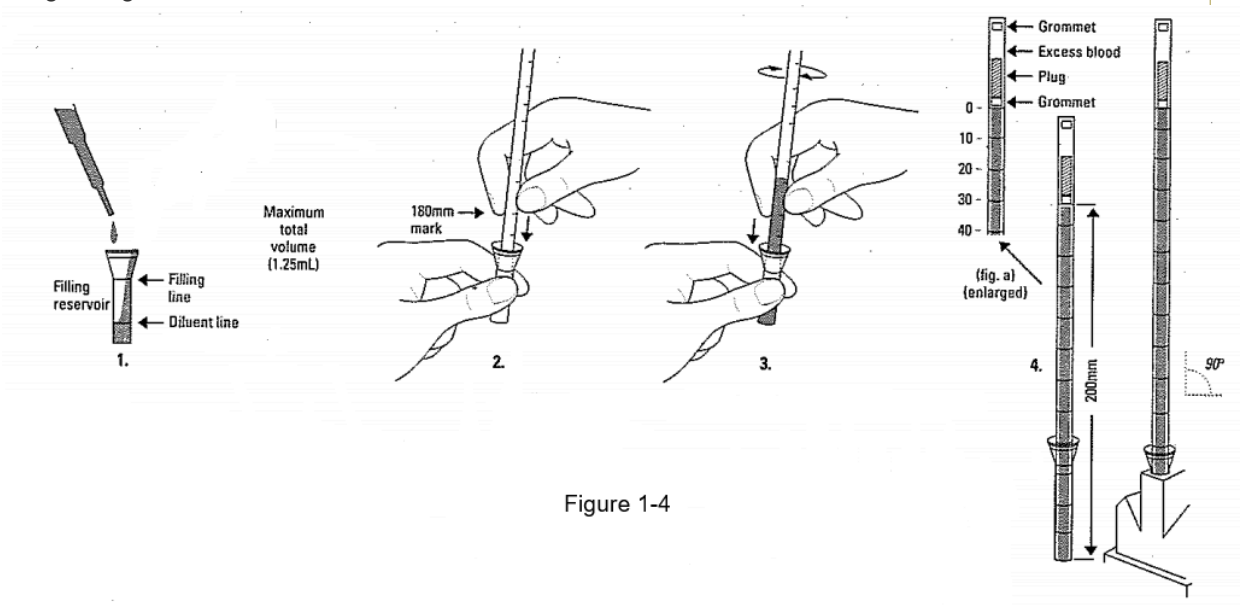

Figure 1-4

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VII. EXPECTED VALUES:

A. Refer to the Hematology Normal Values-RO procedure for current normal ranges.

B. For all elevated Westergren ESRs by Dispette™ method, include the comment: "Result may be falsely elevated if patient has anemia."

VIII. LIMITATIONS:

Anemia may cause a spuriously elevated ESR in some patients. There is no effective method for correcting anemia in the Westergren method. Refer to the departmental policy above for reporting elevated ESR results.

IX. NOTES:

A. Tilting the column accelerates the ESR. The column must be **exactly** perpendicular. An angle of even 3 degrees from vertical may accelerate the ESR by as much as 30 percent.

B. The column must be left **undisturbed**, out of direct sunlight, for **precisely** one hour.

C. The temperature must be kept within 20-25^{o}C.

D. The column of blood must **not** have any air bubbles.

E. If the test is delayed, some samples with elevated ESRs will be falsely low. On standing, erythrocytes tend to become spherical and less readily form rouleaux.

F. Removal of fibrinogen by defibrination (e.g. clot formation) lowers the ESR.

G. Gross hemolysis means fewer RBCs (simulates anemia) and zeta potential change, resulting in an increased

ESR.

H. Red cells with abnormal or irregular shapes (e.g. sickle cells, spherocytes) hinder rouleaux to lower the ESR.

I. Heparin alters the membrane zeta potential and thus cannot be used as an anticoagulant.

J. When documenting Dispette™ patient results, print out the Beaker barcode label and affix to 8½ x 11" sheet of paper. Write Dispette™ results on barcode then place in Dispette™ QC and Patient Results binder after entering results in Beaker.

K. Erythrocyte sedimentation is not linear. The final result must never be estimated before the end of the 60 minute sedimentation period.

X. REFERENCES:

A. Brown B. Hematology: Principles and procedures. Philadelphia: Lea & Febinger, 1984: 52-54.

B. Henry JB. Clinical Diagnosis and Management. 18th ED., Philadelphia: WB Saunders, 1991: 589-590, 599-601.

C. National Committee for Clinical Laboratory Standards. Standardized method for the human erythrocyte sedimentation rate (ESR) test. Villanova, PA, 1977.

D. International Council for Standardization in Haematology, Medical Laboratory Observer, November, 1992.

E. Cardinal Health. Dispette Disposable Pipet, 2003.

F. Thermo Fisher. Dispette™ 2 Saline package insert, issued January 1, 2019.

Attachments

Dispette QC Log.pdf

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	12/17/2021
Hematology Medical Director Designee	Ann Marie Blenc: System Med Dir, Hematopath	12/17/2021
Policy and Forms Steering Committee Approval (if needed)	Michele Sedlak: Medical Technologist Lead	12/14/2021
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	9/30/2021
System Manager	Rebecca Bacarella: Mgr Laboratory	9/30/2021
	Michele Sedlak: Medical Technologist Lead	9/29/2021

Applicability

Royal Oak