

## WESTERGREN SEDIMENTATION RATE USING K<sub>3</sub>EDTA

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### Abstract

**Background:** The (ESR) measures the sedimentation rate of aggregated erythrocyte in plasma, the (ICSH) recommendation published in 1977 on measurement of the ESR was for a selected (routine) method based on that of Westergren.

**Objectives:** This study describes the use of K<sub>3</sub>EDTA as an alternative anticoagulant to sodium citrate in measuring the ESR by the Westergren technique. We have therefore used this method to compare the results of ESR by both the classic and modified methods, in addition, to establish the normal limits for the modified method. And to assess the storage stability of whole blood preserved with K<sub>3</sub>EDTA at 4°C.

**Subjects & methods:** The erythrocyte sedimentation rate (ESR) values determined by the classic and the modified Westergren methods were recorded in 84 persons.

**Results:** There was no significant difference between the two methods using paired t-test ( $0.5 > p > 0.1$ ), with a mean ESR in the classic method of 24.69 mm/hr, and 25.01 mm/hr in the modified Westergren method. Also the normal values of ESR using the modified method had been recorded in 74 apparently healthy persons, women tend to have a higher sedimentation rate values than men of comparable age. The storage stability of ESR of whole blood anticoagulant with K<sub>3</sub>EDTA was determined; there was excellent storage stability up to 12 hours.

**Conclusion:** The modified method is an excellent method for measuring ESR using K<sub>3</sub>EDTA anticoagulant, and it can be used in routine work because using the same anticoagulant used in routine hematological work. There was no significant difference between this method and the classic Westergren method.

**Key Words:** Westergren Sedimentation Rate, K<sub>3</sub>EDTA

Iraqi J Med Sci, 2004; Vol.3(2): 132-135

### Introduction

The erythrocyte sedimentation rate (ESR) test measures the sedimentation rate of aggregated erythrocytes in plasma. The international committee for standardization in hematology (ICSH) recommendation published in 1977 on measurement of the ESR was for a selected (routine) method based on that of Westergren method which proved a greater precision than the Wintrobe method<sup>1-4</sup>. Measurement of the ESR is frequently used non specific test which may indicate the presence of inflammation. The Westergren method for measuring the ESR has greater clinical precision than the Wintrobe method<sup>1-4</sup>.

Measurement of the ESR is a frequently used non-specific test which may indicate the presence of inflammation or occult disease and confirm the presence of disease diagnosed by other means, or serve as a guide in following the course of a disease.

The ESR may be significantly increased suggesting an organic disease, when clinical and

other laboratory findings are negative. Conversely, a normal ESR is reassuring in a patient believed to have no organic disease, although a normal ESR does not rule out the presence of organic disease<sup>5</sup>.

This study described the use of K<sub>3</sub> EDTA as an alternative anticoagulant to sodium citrate in measuring the ESR by the Westergren technique following the exact procedure of the classic method.

### Aim of the study:

This study is designed to:

1. Compare the results of ESR read by both the classic and modified methods.
2. Establishing the normal limits for the modified method.
3. Assessing the storage stability of whole blood preserved with K<sub>3</sub>EDTA at 4°C.

### Patients & Methods

Prospectively, during the period from February 2001 to March 2002, the blood samples of 84 patients with different clinical disorders (42 males and 42 females, their ages range between 10-75 years) along with 74 apparently healthy individuals, no systemic illness, non pregnant and on no medications, utilized as control (37

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Received 8<sup>th</sup> December 2002; Accepted 30<sup>th</sup> March 2004.

males and 37 females, their ages range between 10-73 years), were analyzed for ESR measurement by the classic and modified Westergren methods at the Hematological department of the Teaching Laboratories of College of Medicine, Al-Nahrain University.

**Procedure**

*Classic Westergren method<sup>4</sup>*

1. Four parts of venous whole blood is diluted with one part of 3.8% (W/V) of sodium citrate.
2. The sample is well mixed and blood drawn up into Westergren tube to 200 marks.
3. The tube is placed exactly vertical and left undisturbed for 60 minutes, free from vibration and away from direct sun light.
4. The height of plasma above the upper limit of the column of sediment cells is then read as the ESR.

*The modified Westergren method<sup>16</sup>*

Its procedure and limitations are similar to the above method with the exception that, in this method 4 parts of the anticoagulated blood by 30% (W/V) K<sub>3</sub>EDTA is diluted with 1 part of 0.85% (W/V) saline solution.

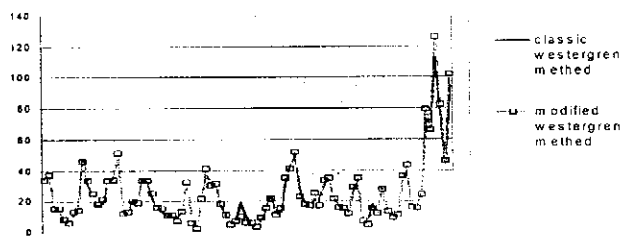
*Statistical analysis*

Using paired t-test for difference between means of paired samples. The values were expressed as means ± SD, when the P value (0.5>P>0.1), there is no significant difference between the two means.

**Results**

Listed in table 1 are E.S.R. values determined by means of the classic and the modified Westergren methods in 84 persons. There was no significant difference between the two methods (0.5>p>0.1). The mean E.S.R. with the classic Westergren method is 24.69 mm/hr and the mean E.S.R. with the modified Westergren method is 25.01 mm/hr. The E.S.R. values range from 2mm/hr to 126mm/hr. When the ESR is greater than 25 mm/hr, the results by the modified method tends to be slightly higher than the classic method as shown in the figure.

Comparison Between Classical & Modified Methods for E.S.R Estimation



No.	Classic westergren method	Modified westergren method	Differences d
1	35	34	-1
2	37	37	0
3	16	15	-1
4	15	15	0
5	7	8	+1
6	6	6	0
7	12	13	+1
8	15	14	-1
9	48	46	-2
10	31	33	+2
11	25	25	0
12	17	18	+1
13	19	21	+2
14	33	33	0
15	35	34	-1
16	50	51	+1
17	12	12	0
18	13	13	0
19	21	20	-1
20	19	19	0
21	35	33	-2
22	33	33	0
23	22	25	+3
24	16	16	0
25	13	15	+2
26	11	11	0
27	10	11	+1
28	9	7	-2
29	13	13	0
30	32	32	0
31	7	6	-1
32	2	2	0
33	22	21	-1
34	40	41	+1
35	30	30	0
36	31	31	0
37	17	18	+1
38	11	11	0
39	5	5	0
40	7	7	0
41	19	18	-1
42	11	10	-1
43	5	6	+1
44	3	3	0
45	8	9	+1
46	15	15	0
47	23	21	-2
48	11	11	0
49	13	15	+2
50	33	35	+2
51	42	41	-1
52	50	52	+2
53	26	23	-3
54	18	18	0
55	17	17	0
56	21	25	+4
57	20	17	-3
58	31	33	+2
59	35	35	0
60	22	21	-1
61	16	16	0
62	15	15	0
63	12	12	0
64	27	29	+2
65	34	35	+1
66	7	7	0
67	5	5	0
68	18	15	-3
69	12	12	0
70	28	27	-1
71	13	13	0
72	9	9	0

Table 1 (continued)

No.	Classic westergren method	Modified westergren method	Differences d
73	11	11	0
74	35	36	+1
75	41	43	+2
76	16	16	0
77	15	15	0
78	25	24	-1
79	75	79	+4
80	68	66	-2
81	118	126	+8
82	80	82	+2
83	44	46	+2
84	95	102	+7
Mean	24.69	25.01	
S.D.	20.003	20.945	
P value > 0.1 N.S.			

Listed in table 2 are E.S.R. values for 37 apparently normal males and 37 apparently normal females. The results are ranked in order of age for both sexes. Females tend to have higher sedimentation values than males of comparable age, and the normal E.S.R. tends to increase slightly with age.

Table 2: Normal ESR values for 74 healthy individuals

Age (years)	Men mm/hr	Women mm/hr
18-30	6.8	10.3
31-40	8.6	11.6
41-50	10.8	13.0
51-60	12.0	18.5
> 60	14.1	19.7

Table 3 is a tabulation of E.S.R. values of 31 samples after storage for 0,8,12, and 16 hours at 4°C. There is an excellent storage stability up to 12 hours and there is no significant difference between the mean ESR at 0,8,12 hours (0.5 >P> 0.1).

Table 3: Storage stability of the modified westergren E.S.R. at different temperatures in 31 samples

No.	Immediate mm/hr	8 hours mm/hr	12 hours mm/hr	16 hours mm/hr
1	2	3	2	2
2	3	5	5	4
3	4	4	5	6
4	4	5	4	6
5	4	6	7	5
6	6	5	4	6
7	6	5	6	5
8	6	7	8	4
9	7	8	8	8
10	9	10		10
11	10	10	10	9
12	10	10	11	9
13	11	10	12	10
14	12	14	15	13
15	12	14	14	5
16	14	14	16	16
17	26	26	28	21
18	26	25	28	21

Table 3 (continued)

No.	Immediate mm/hr	8 hours mm/hr	12 hours mm/hr	16 hours mm/hr
19	30	32	34	23
20	31	34	39	32
21	31	31	31	20
22	35	36	38	30
23	50	47	50	46
24	65	67	68	60
25	68	70	75	51
26	70	70	68	48
27	70	70	70	68
28	77	79	83	78
29	83	81	85	79
30	101	98	105	95
31	103	107	118	92

Discussion

The excellent correlation between the results of classic and modified Westergren methods makes it unnecessary to have special tubes filled with 3.8 percent (w/v) of sodium citrate for collection and dilution. Dilution with saline solution in the laboratory rather than with sodium citrate at the time of collection will conserve patient's blood because the K<sub>3</sub>EDTA tubes can be used for other hematology and chemistry tests<sup>6-8</sup>. Blood samples may be stored up to 12 hours at 4°C before dilution with saline without significantly altering the ESR (table 3), which makes it possible for a busy laboratory to schedule its work. Moreover, EDTA is an ideal anticoagulant in that it has the least effect on blood cell morphology and does not interfere with electronic counting of red cells and leukocytes and platelets<sup>1,2,9-14</sup>.

The result of this study of normal values (table 2) is in agreement with Hilder and Gunz<sup>15</sup>. These authors studied 603 healthy blood donors of varying age, using the classic Westergren method.

Dawson believed the normal range to be 0 to 10 mm. per hr. for men and 0 to 15 for women. Westergren originally believed the normal range to be 0 to 5 mm. per hr. for men and 0 to 10 for women, but he did not regard readings as abnormal until they exceed 10 mm/hr in men and 20 mm/hr in women.

Blood anticoagulated with tripotassium ethylenediamine tetraacetate monohydrate (K<sub>3</sub>EDTA) and subsequently diluted with 0.85 percent (W/V) saline solution (4 parts of whole blood with one part of saline solution has been shown to yield an excellent ESR values compared to classic Westergren method. The samples remain stable up to 12 hour without affecting the rate of red cell sedimentation.

Moreover, preserving blood in EDTA tubes will preserve the samples for other haematologic and biochemical tests that patients may need avoiding repuncturing.

### **Conclusions**

1. The modified method is an excellent method for measuring ESR using K<sub>3</sub>EDTA anticoagulant.
2. This method can be used in routine work using the same anticoagulant (K<sub>3</sub>EDTA) used in routine Hematological work.
3. There was no significant difference between this method and the classic Westergren method.

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