Correlation Study: Greiner Bio One SRT10/II, SRS20/II, SRS100/II 15 minutes reading compared to 1 hour Manual Westergren Method

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Abstract
The manual Westergren method is considered the reference method for evaluation of ESR analysis performances, as described by the Clinical and Laboratory Standard Institute® in document H02-A5. In order to evaluate a new reading time integrated in Greiner instruments, a correlation study between the Westergren manual method and Greiner instruments was performed. The aim of this study is to assess acceptable correlation between the manual Westergren and the automatic reading at 15 minutes performed by the Greiner instruments.

Introduction[1]
The erythroctyte sedimentation rate analysis was first described in 30s years, and it is still one of the most widely performed laboratory tests. The Westergren method to measure the ESR has remained unchanged since its inception and was recommended as the method to be chosen by the International Council for Standardization in Haematology (ICSH) in 1973 and 1977.
Erythrocyte sedimentation is a nonspecific reaction; it is a measure of the presence and severity of pathological processes. In general the ESR is increased in acute, general infections and in localized, acute, inflammatory conditions. The usefulness of ESR is evaluating patients with unexplained symptoms when inflammation and infectious disease are suspected and a specific diagnosis is not available effectively using other tests.
ESR analysis is described by a typical sigmoid curve in which 3 phases can be distinguished; “lag phase”, time during which the red cells form rouleaux, “decantation phase”, time during which rouleaux fall more rapidly, “packing phase”, time during which the rouleaux pile up on the bottom of container. The size of the rouleaux is critical for the outcome of sedimentation; in facts the rate of aggregation is a consequence of the reciprocal effect between the red cells membrane surface and some plasma proteins. They have a high affinity for the red cells membrane glycoproteins, and a sufficient molecular size to form bridges between red cells themselves. Examples of these plasma proteins are Fibrinogen, IgM, α_2-macroglobulin.
The manual ESR analysis is performed in 60 minutes. The reported numerical value, in mm/h, is the distance from the bottom of the surface meniscus to the top of the red cells sediment in a column of anticoagulated blood that has remained perpendicular in a special-purpose pipette. The pipette can be made by glass or plastic, colorless, with an internal diameter greater than 2.55 mm, and a height of 200 mm. It must have a measuring scale with steps of 1 mm, and it must be kept in perfectly vertical position throughout the analysis time. The blood samples must be not clotted; the suitable anticoagulant solutions are the EDTA (potassium or sodium) in final concentration 3.5 – 5.4 mmol/L, the sodium citrate in ratio 1:4 with whole blood, the saline solution in ratio 1:4 with whole blood. ESR test must be performed within 4 hours from sampling if samples are stored at room temperature.
The ESR instrument SRT10/II, SRS20/II, SRS100/II designed by Vital Diagnostics for Greiner Bio One using VACUETTE® tubes are automatic ESR analyzers which perform the ESR test in 30 minutes and 15 minutes, and give a final result in mm/h, correlated to the manual Westergren method. The SRT10/II is a ESR instrument with 10 independent reading channels, the SRS20/II has 20 independent reading channels, the SRS100/II has 100 independent reading channels. Every channel reads the sample randomly, using infra-red beams which detect precisely the height of blood column inside tube. The design of instruments assures the perfect vertical position of tubes, and the integrated temperature sensor, enables standardization of the final ESR results to a reference temperature of 18°C (Manley[2]).
The VACUETTE® is an evacuated blood collection tube 9 mm in diameter and height of 120 mm. The vacuum inside tube assures an aspiration of 1.6mL of whole blood; the volume of sodium citrate contained assures the correct ration 1:4 with sample. The tube containing sample has to be simply inserted into 1 reading channel of ESR instruments to start the ESR analysis. After 30 minutes the instruments automatically display the final ESR result expressed in mm/h. After 15 minutes the instruments display a pre-indication of the result expressed in mm/h.

Materials

The materials used for this correlation study were:

- *SRT10/II instrument*

Automatic random access ESR analyzer with 10 reading channels, able to perform the ESR analysis in 15 and 30 minutes, giving results in mm/h.

- *SRS20/II instrument*

Automatic random access ESR analyzer with 20 reading channels, able to perform the ESR analysis in 15 and 30 minutes, giving results in mm/h.

- *SRS100/II instrument*

Automatic random access ESR analyzer with 100 reading channels, able to perform the ESR analysis in 15 and 30 minutes, giving results in mm/h.

- *Duo-Mix*

Automatic mixer for 9 mm and 12/13 mm tubes.

- Greiner Bio One VACUETTE®*vacuum tubes*

ESR vacuum tubes of 9 mm diameter and 120 mm of height. Tubes are made in glass and contain 1.6 mL of sample. The sodium citrate inside, used as anticoagulant, assures the ratio 1:4 with whole blood.

- *Primary tubes*

ESR vacuum tubes of 12 mm diameter, suitable for manual ESR analysis, containing sodium citrate as anticoagulant, in volume that assures the ratio 1:4 with whole blood. Tubes are made of plastic and contain 2.5 mL of sample.

- *Westergren pipettes*

Plastic pipettes for manual Westergren method, scaled until 200 mm.

- *Holder*

Plastic holder for manual Westergren pipettes, that assures the correct vertical position during analysis.

- *Timer*

Digital timer to measure 60 minutes for reading the final Westergren result.

Specimens

Samples used are whole blood samples, taken from patients from Opera Santa Teresa, Reparto Lunga Degenza, in Ravenna, Italy.

2 blood samples were obtained from each patient using the venipuncture technique for primary tube for manual Westergren ESR analysis, VACUETTE®. All samples were analyzed within 4 hours from sampling.

Methods[3]

From each patient 2 tubes were collected, 1 tube for manual method, 1 VACUETTE® tube for SRT10/II, SRS20/II and SRS100/II instruments. They were labeled as same patient using the same progressive number. Then they were transferred to the Laboratory of Opera Santa Teresa and immediately mixed, using Duo-Mix device. The primary tubes and VACUETTE® tubes were mixed for 5 minutes in F3 and S2, in order to assure the correct re-suspension of red cells.

At the end of mixing phase, samples for manual method were aspirated in 200 mm plastic pipettes, and placed into the dedicated rack. Timer was started and result was read after 60 minutes. Samples for SRT10/II, SRS20/II, SRS100/II were loaded directly into the reading channel corresponding to the assigned progressive number. Since for manual method it was not possible to convert the final result considering the reference temperature of 18°C, this function on all of the ESR instruments was disabled.

ESR instruments were also connected to thermal printers, in order to obtain an automatic print out of results. Manual ESR results were recorded in a dedicated data sheet.

All the ESR analyses were performed in duplicate.

Statistical Analysis[4]

The obtained results were processed using the following statistical methods.

Linear regression

All data were plotted putting the manual method in x axis and SRT10/II, SRS20/II or SRS100 results in y axis. A linear curve was used to fit points and slope and R^2 were evaluated.

Correlation coefficient

The correlation coefficient calculated as follow was evaluated:

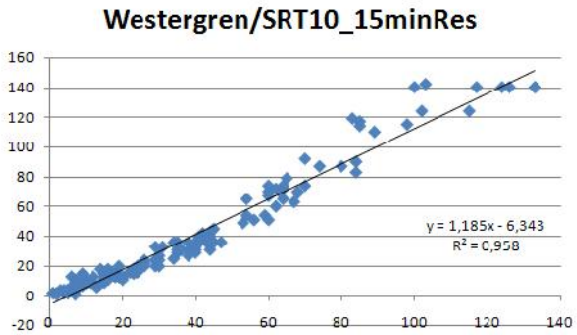
$$r = \frac{[\sum(x_1 - \mu_{x1})(x_2 - \mu_{x2})]}{\sqrt{[\sum(x_1 - \mu_{x1})^2][\sum(x_2 - \mu_{x2})^2]}}$$

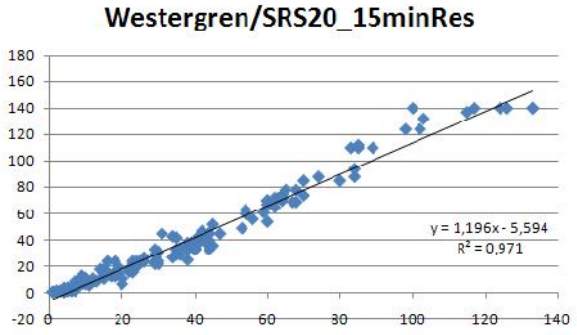
Results after 15 minutes reading

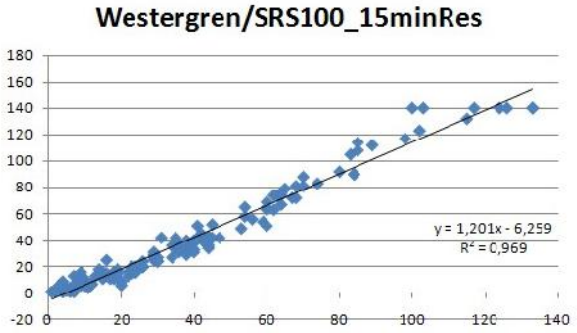
Data were collected in a range from 0 – 140 mm/h, distributed in 4 ranges in this way:
79 samples from 0 – 30 mm/h
39 samples from 31 – 60 mm/h
20 samples from 61 – 90 mm/h
10 samples higher than 91 mm/h
Total: 148 samples
Raw data are reported below:

N.	Westergren	SRT10/II	SRS20/II	SRS100/II
1	1	2	1	1
2	1	2	1	1
3	2	2	2	2
4	2	1	1	1
5	3	4	2	6
6	4	4	4	9
7	4	2	1	1
8	4	2	1	1
9	5	4	4	4
10	5	2	2	4
11	5	2	2	2
12	5	4	4	2
13	6	13	4	4
14	6	4	4	4
15	6	4	2	1
16	6	6	2	2
17	6	4	2	2
18	7	11	2	1
19	7	4	4	4
20	7	1	2	1
21	7	4	6	6
22	7	9	9	13
23	8	6	6	9
24	8	9	7	6
25	8	7	6	6
26	9	13	11	13
27	9	11	11	9
28	9	15	13	16
29	9	7	7	6
30	9	13	11	7
31	10	11	11	11
32	10	9	11	9
33	10	9	7	7
34	10	9	11	6
35	11	9	6	4
36	12	13	9	7
37	12	9	11	7
38	13	6	9	13
39	14	18	18	18
40	15	9	18	16
41	15	13	15	13
42	15	15	11	11
43	15	11	13	13
44	16	18	24	25
45	16	11	15	15
46	17	15	13	13
47	17	15	13	11
48	18	16	24	16
49	18	15	16	15
50	18	13	13	13
51	18	13	13	11
52	19	13	16	15
53	19	13	15	11
54	19	20	20	18
55	19	13	15	16
56	20	13	7	6
57	20	11	11	9
58	22	16	18	13
59	22	16	16	16
60	23	18	18	18
61	23	18	24	20
62	23	15	16	16
63	23	16	16	15
64	23	16	22	16
65	23	18	18	20
66	24	16	24	16
67	24	18	20	16
68	24	20	22	16
69	24	16	20	20
70	26	22	27	24
71	26	24	24	20
72	26	20	24	20
73	29	24	24	27
74	29	20	22	25
75	29	24	29	29
76	29	33	33	31
77	30	24	31	24
78	30	27	24	27
79	30	20	22	24
80	31	33	45	42
81	34	25	27	27
82	34	36	43	36
83	35	29	29	33
84	35	36	42	42
85	35	31	33	34
86	36	31	29	31
87	36	31	34	36
88	38	36	38	40
89	38	31	33	33
90	38	27	25	29
91	39	33	38	34
92	39	33	38	33
93	40	31	33	33
94	40	36	34	38
95	40	33	38	40
96	40	29	33	31
97	41	40	43	51

98	41	36	38	42
99	42	42	47	45
100	43	36	38	38
101	43	38	40	40
102	44	31	33	34
103	44	43	45	40
104	44	36	38	36
105	44	40	45	40
106	44	38	40	43
107	45	45	52	52
108	45	36	36	42
109	47	36	45	42
110	53	49	49	49
111	54	65	63	65
112	54	54	60	58
113	56	51	56	56
114	59	54	61	54
115	60	51	54	51
116	60	67	70	63
117	60	70	70	69
118	60	74	67	65
119	62	72	72	74
120	62	60	65	63
121	63	70	72	74
122	64	72	70	76
123	64	74	74	74
124	64	65	72	67
125	65	79	78	79
126	67	63	69	72
127	68	69	69	72
128	68	70	78	81
129	70	92	85	88
130	70	74	74	81
131	74	87	88	83
132	80	87	85	92
133	83	119	110	105
134	84	90	88	90
135	84	83	94	90
136	85	117	110	108
137	85	114	112	114
138	89	110	110	112
139	98	115	124	117
140	100	140	140	140
141	102	124	124	123
142	103	142	132	140
143	115	124	137	132
144	117	140	140	140
145	124	140	140	140
146	126	140	140	140
147	133	140	140	140
148	133	140	140	140







Equation of linear curve is reported below:
Westergren versus SRT10/II
$y = 1.185x – 6.343$
$R^2 = 0.958$

Westergren versus SRS20/II
$y = 1.196x – 5.594$
$R^2 = 0.971$

Westergren versus SRS100/II
$y = 1.201x + 6.259$
$R^2 = 0.969$

The acceptability criteria were: $R^2 \geq 0.90$

Results after 30 minutes reading

Data were collected in a range from 0 – 140 mm/h, distributed in 4 ranges in this way:

79 samples from 0 – 30 mm/h

39 samples from 31 – 60 mm/h

20 samples from 61 – 90 mm/h

10 samples higher than 91 mm/h

Total: 148 samples

Raw data are reported below:

N.	Westergren	SRT10/II	SRS20/II	SRS100/II
1	1	2	1	1
2	1	2	2	1
3	2	2	2	2
4	2	2	2	2
5	3	2	3	4
6	4	4	6	6
7	4	2	2	2
8	4	2	2	2
9	5	4	5	5
10	5	2	3	4
11	5	2	3	4
12	5	3	4	3
13	6	2	4	5
14	6	3	4	3
15	6	2	2	2
16	6	5	5	4
17	6	3	3	4
18	7	8	5	3
19	7	5	5	3
20	7	2	3	2
21	7	3	5	6
22	7	8	8	10
23	8	6	7	8
24	8	8	8	8
25	8	5	6	8
26	9	14	13	13
27	9	12	12	13
28	9	14	14	15
29	9	7	8	7
30	9	9	9	10
31	10	12	12	11
32	10	9	10	9
33	10	8	9	8
34	10	11	13	10
35	11	8	8	8
36	12	9	9	10
37	12	10	11	9
38	13	10	12	13
39	14	19	18	20
40	15	10	14	14
41	15	14	14	14
42	15	10	9	9
43	15	13	14	14
44	16	15	19	21
45	16	14	14	14
46	17	16	15	17
47	17	16	14	15
48	18	18	20	20
49	18	15	16	19
50	18	15	14	15
51	18	14	14	14
52	19	16	18	18
53	19	18	19	18
54	19	23	22	22
55	19	16	16	20
56	20	10	9	10
57	20	12	14	14
58	22	20	21	21
59	22	20	19	21
60	23	19	20	22
61	23	24	27	27
62	23	20	20	20
63	23	19	18	19
64	23	20	23	22
65	23	20	20	22
66	24	21	23	23
67	24	23	24	23
68	24	26	27	26
69	24	20	21	22
70	26	28	31	31
71	26	28	27	25
72	26	25	26	25
73	29	31	32	36
74	29	23	24	28
75	29	28	32	31
76	29	38	37	34
77	30	28	31	29
78	30	31	29	31
79	30	24	25	28
80	31	49	53	51
81	34	31	31	31
82	34	45	46	42
83	35	33	33	34
84	35	49	53	51
85	35	36	37	37
86	36	35	35	34
87	36	37	38	38
88	38	40	40	40
89	38	37	37	36
90	38	31	31	34
91	39	41	44	42
92	39	39	41	39
93	40	37	38	38
94	40	41	41	44
95	40	41	42	41
96	40	35	37	35
97	41	51	53	54

98	41	41	42	45
99	42	48	49	48
100	43	41	41	41
101	43	44	45	42
102	44	35	37	37
103	44	47	48	46
104	44	41	42	41
105	44	45	47	46
106	44	45	46	47
107	45	49	53	55
108	45	49	47	52
109	47	44	47	46
110	53	53	53	52
111	54	65	64	65
112	54	60	62	62
113	56	59	61	60
114	59	60	64	61
115	60	53	53	53
116	60	78	81	79
117	60	78	75	73
118	60	74	68	66
119	62	71	71	75
120	62	65	67	65
121	63	77	78	78
122	64	73	71	76
123	64	79	79	78
124	64	74	76	71
125	65	81	79	79
126	67	66	68	71
127	68	67	67	71
128	68	76	79	83
129	70	95	91	91
130	70	75	76	81
131	74	92	91	90
132	80	85	85	89
133	83	107	104	101
134	84	92	92	92
135	84	85	87	87
136	85	107	104	103
137	85	105	104	104
138	89	104	103	103
139	98	114	112	112
140	100	137	134	137
141	102	115	115	116
142	103	118	119	112
143	115	118	118	119
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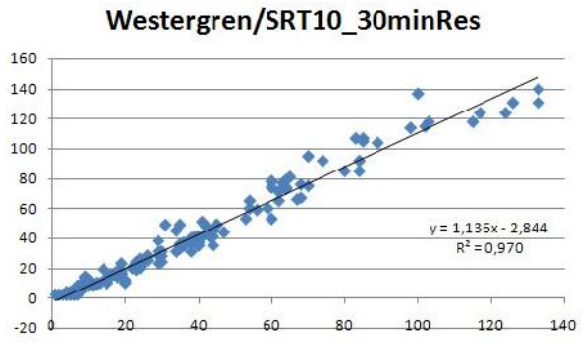

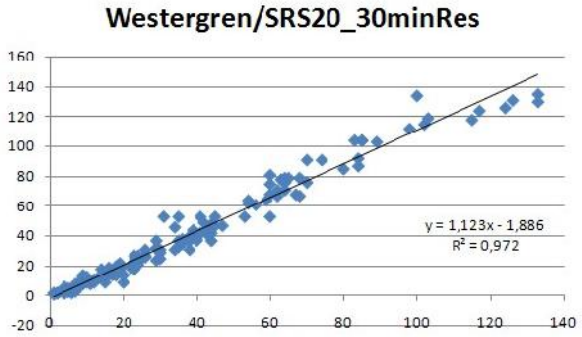

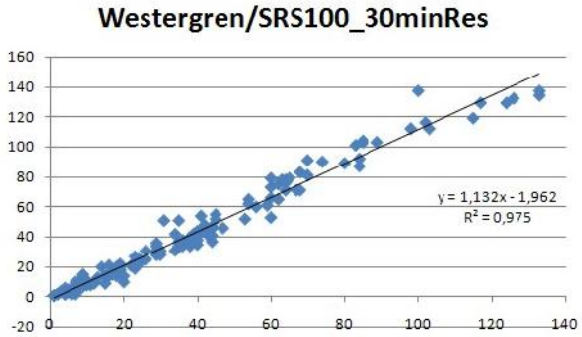

Equation of linear curve is reported below:
Westergren versus SRT10/II
$y = 1.136x – 2.844$
$R^2 = 0.970$

Westergren versus SRS20/II
$y = 1.123x – 1.886$
$R^2 = 0.972$

Westergren versus SRS100/II
$y = 1.132x + 1.962$
$R^2 = 0.975$

The acceptability criteria were: $R^2 \geq 0.90$

Conclusion

The presented study demonstrates that the automated ESR methods (SRT10/II, SRS20/II or SRS100 analyzers) give equivalent results with reference to the standard Westergren manual method both for the 15 minutes reading and the 30 minutes reading.

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