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Three-Way Comparison of Methods for the Measurement of the Erythrocyte Sedimentation Rate

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An erythrocyte sedimentation rate (ESR) is a nonspecific sickness index, which is not diagnostic of any particular disease, but which when elevated may indicate the presence of inflammation, infection, rheumatologic disease, or neoplasm. Technological advances continue to evolve to make this old test to conform to requirements of a modern analytical laboratory. In this evaluation, two new semi-automated ESR measuring systems, the HumaSed and ESR-Auto Plus® (EAP), were compared with the Westergren method with regard to the accuracy and precision of ESR measurement, sample stability, and interference of various substances with the ESR assay. Samples from 125 patients were analyzed with the three methods and the results compared using linear regression and Bland–Altman plots. The mean ESR values of the HumaSed (32.10 ± 4.86) and EAP (38.09 ± 5.33) were comparable to that of the Westergren (31.54 ± 4.94). The high correlation coefficients of 0.910–0.96 and the Bland–Altman scatter plots revealed good association and agreement between the three methods. Bias between the three methods was small and the imprecision was within acceptable limits. ESR analysis beyond 4 hr was found to be unacceptable owing to sample instability. There was bilirubin and lipid but not heparin interference in the two automated systems. Overall, two automated analyzers were found to be fast, reliable, standardized, simplified, and safe instruments with accuracy and precision for ESR measurement comparable to the Westergren. J. Clin. Lab. Anal. 22:346–352, 2008.

Key words: ESR; method; comparison; HumaSed; ESR-Auto Plus®; evaluation; validation

INTRODUCTION

The erythrocyte sedimentation rate (ESR) test measures the distance in millimeters that erythrocytes fall during a specified time period as a function of acute phase proteins and cellular composition of blood (1–3). It is a nonspecific sickness index, which is not diagnostic of any particular disease. However, an elevated ESR may indicate the presence of inflammation, infection, rheumatologic disease, or neoplasm in patients who are not well. A normal ESR has a strong negative predictive value for these conditions. Measurement of the ESR has diagnostic value in some conditions (4,5) and allows monitoring of therapeutic interventions in others (6,7).

The modified Westergren manual method is the reference standard for ESR measurement (2,8). In the modern clinical laboratory, the Westergren method has a number of shortcomings in the ESR measurement, which include the relatively slow turnaround time, inaccurate results that may occur owing to incorrect positioning of the ESR tube, errors in the manual reading of the tube, lack of analytical standardization, and the influence of the surrounding temperature on the ESR (9). The sample volumes required for the test are relatively large (10) and the open tube system of the Westergren manual method may confer a risk of possible exposure of the operator to biological infectious hazards (3,11).

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Recently, a number of automated and semi-automated ESR measurement systems have evolved to address some of the above Westergren method limitations (7,9,12–14). The newer ESR measurement systems have the potential advantages of requiring smaller volumes of sample for analysis (1 ml in some systems), improved speed of testing (15–30 min as opposed to 1 hr), analytical standardization (analytical procedure performed in a single chamber), simplification of testing procedure, as well as a closed testing mode that reduces the risk of exposure to potential infectious agents. These instruments run commercial controls that allow participation in proficiency testing programs.

The HumaSed 40 instrument (Human, Wiesbaden, Germany) is a random access ESR analyzer, which uses the light-emission diode optical system for simultaneous ESR measurement in a number of samples. ESR measurements can be performed from both vacuum and nonvacuum primary sampling citrated tubes. The instrument has a choice of 24 or 48 min analytical time and up to 40 ESR analyses can be performed with uninterrupted sample loading (15).

The ESR-Auto Plus® (EAP) from Streck (Streck, Omaha, NE) is also a semi-automated analyzer using a system of infrared diodes and sensors for the ESR measurement. ESR analysis is performed from citrated vacuumed tubes in the analytical time of 30 min (16). Both the HumaSed 40 and EAP have been calibrated against Westergren and give results in mm/hr.

The aim of this study was to evaluate the HumaSed 40 and the EAP analyzers and to compare their ESR analytical performance with the modified Westergren method currently used in our laboratory. This evaluation was performed, as far as practical, according to the recommendation of the International Committee on Standardization in Haematology (2) as well as the method comparison (17) and ESR reference standards (8) from the Clinical and Laboratory Standards Institute (USA).

MATERIALS AND METHODS

Patient Samples and Controls

The study participants were adult patients attending the outpatient clinics or admitted in the internal medicine units of the Johannesburg Hospital, Gauteng province, South Africa. This study was approved by our local university ethics committee. Informed consent was obtained from all patients prior to study sample collection. Blood collection from patients for the study was done at the same time as venous blood sampling for other diagnostic tests required for patient management. An EDTA and a citrate sample were collected from each consenting patient and collected samples were delivered to the laboratory immediately and analyzed within 4 hr after collection.

Two levels of commercial ESR controls with assigned values were used in this evaluation. For the HumaSed instrument, the Bio-Rad Liquichek™ sedimentation rate levels 1 and 2 controls were used (Bio-Rad Laboratories, Johannesburg, South Africa). The Liquichek™ control is reported to have an open vial stability of 31 days at 18–30°C and a 1-year shelf life at 2–8°C. For the EAP analyzer, the Streck ESR-Chex sedimentation rate levels 1 and 2 controls were used (Streck, ne). The ESR-Chex is reported to have an open vial stability of 95 days at 18–25°C and a closed vial stability of 1 year at 2–10°C. The controls were handled and used as per manufacturer's instructions.

Instruments

The instrument setup, calibration, and analytical optimization were performed by the local supplier's technical staff. Our laboratory staffs were trained on the instrument operation, sample analysis, maintenance, and operational troubleshooting. All reagents and tubes used during instrument calibration as well as control samples were supplied by the respective manufacturers.

Analyses

Analysis of the patient EDTA sample tube was done in replicate according to our established modified Westergren standard operating procedure. The patient sample collected in citrate was split into two, with one sample analyzed in replicate in the HumaSed 40 analyzer and the second sample analyzed in the EAP instrument. ESR analysis was performed in batches in parallel by the same dedicated technologist using all three methods.

Correlation studies

ESR measurements were performed on 375 samples from 125 patients (125 EDTA and 250 citrate split samples) using all three methods. Linear regression and Bland–Altman analyses were performed to measure agreement between the manual and automated methods. The Stata™ 10 (Statacorp, College Station, TX) and Analyze-it™ version 2.07 (Analyze-it Ltd., Leeds, UK) statistic softwares were used for computation and graphical representation of data. Each instrument's analytical range was established from the samples analyzed.

Precision analyses

Within-run precision with control samples

Within-run imprecision was determined using ESR levels 1 and 2 control samples. Each of the control

samples was analyzed ten times by each instrument and the Westergren method. Summary statistic calculated from the measured values was presented in tabular and graphical formats. As large volume of patient sample would have been required for this analysis, only control but no patient samples were analyzed.

Between-run precision with control samples

Between-run imprecision was determined using commercial ESR controls 1 and 2 samples, which were analyzed daily for 10 days to look at the potential calibration drift over time. Summary statistic calculated from the measured values was presented in tabular and graphical formats.

Sample stability studies

The ESR of two randomly selected patient samples was measured by each of the analyzers at 4, 6, and 24 hr after collection. Samples were stored at 4°C between the analyses. The ESR results were summarized in tabular form and graphically represented.

TABLE 1. Summary Statistics of ESR Analyses Using the Three ESR Methods

	n	Min	Max	Mean (95% CI)	SEM	SD	CV
Westergren	125	1	121	31.54 (±4.94)	2.52	28.17	89.34
HumaSed	125	1	125	32.10 (±4.86)	2.48	27.71	86.34
EAP	125	1	110	38.09 (±5.33)	2.72	30.43	79.9

ESR, erythrocyte sedimentation rate; Min, minimum; Max, maximum; CI, confidence interval; SEM, standard error of mean; SD, standard deviation; CV, coefficient of variation; EAP, ESR-Auto Plus®.

Interference studies

The ESR of six randomly selected patient samples was measured by the two automated methods. Two samples were then spiked with total parenteral nutrition solution, two with hemolysate, and the third pair with heparin as previously described (18). Repeat ESR analyses were performed and the results before and after sample spiking were tabulated and graphically represented.

RESULTS

Correlation Studies

Data from the correlation studies are reported as summary statistics in Tables 1–3 and Figures 1 and 2.

Precision Study Results

Precision study results for the measurement of ESR levels 1 and 2 controls using the HumaSed and EAP instruments are given in Table 4.

Sample Stability Study Results

Results of stability studies done on the HumaSed 40 and EAP analyzers at 0, 4, 6, and 24 hr are given in Table 3 (Fig. 3).

Interference Study Results

The inference study results are shown in Figure 4. The presence of lipids appears to cause a falsely low ESR measurement in both instruments. Although no definite trend is apparent with the presence of bilirubin in the samples, the results are clearly unreliable. Heparin has

TABLE 2. Correlation Data Comparison of the Three ESR Methods

	n	*r*	SE	r^2	95% CI
A. Correlation coefficient comparison					
Westergren/HumaSed comparison	125	0.93	10.3	0.86	0.90–0.95
Westergren/EAP comparison	125	0.96	8.8	0.92	0.94–0.97
HumaSed/EAP comparison	125	0.91	13	0.82	0.88–0.94
	n	Intercept	SE	95% CI	
B. Intercept comparison					
Westergren/HumaSed comparison	125	3.3	1.39	0.54–6.05	
Westergren/EAP comparison	125	5.46	1.19	3.12–7.81	
HumaSed/EAP comparison	125	6.18	1.78	2.65–9.67	
	n	Slope	SE	95% CI	
C. Slope comparison					
Westergren/HumaSed comparison	125	0.91	0.033	0.85–0.98	
Westergren/EAP comparison	125	1.03	0.03	0.98–1.09	
HumaSed/EAP comparison	125	0.99	0.04	0.91–1.08	

ESR, erythrocyte sedimentation rate; *r*, correlation coefficient; SE, standard error; r^2, variance; CI, confidence interval; EAP, ESR-Auto Plus®.

TABLE 3. Summary Data of Difference Comparison Between the Three Methods

	n	95% LOA	Bias	SE	95% CI	*P* value
Westergren–HumaSed	125	−20.2 to 21.3	0.60	0.95	−3.7 to 1.2	0.56
Westergren–EAP	125	−10.7 to 23.8	6.60	0.79	5.0–8.1	<0.0001
HumaSed–EAP	125	−19.3 to 31.3	6.00	1.16	3.7–8.3	<0.0001

EAP, ESR-Auto Plus®; ESR, erythrocyte sedimentation rate; LOA, level of agreement; SE, standard error; CI, confidence interval.

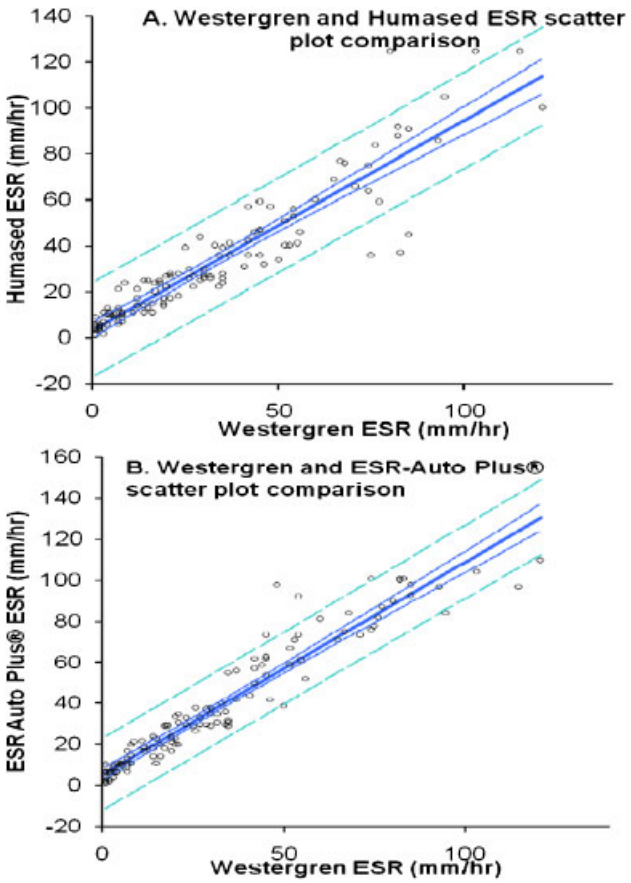

Fig. 1. Westergren, HumaSed, and ESR-Auto Plus® correlation scatter plots. The open circles are the ESR data points, the solid lines are the linear fit, the dotted lines are the 95% CI, and the dashed lines are the 95% prediction interval. In the HumaSed plot (**A**), the intercept of the fit line is 0.9121 and the slope is 3.296. In the ESR-Auto Plus® (**B**) plot, the slope of the fit line is 1.034 and the intercept is 5.468. ESR, erythrocyte sedimentation rate.

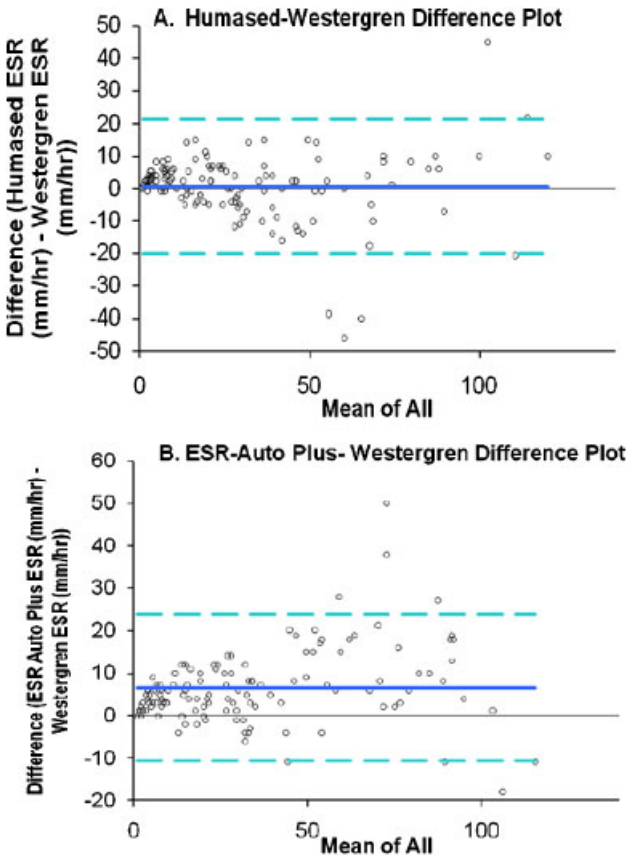

Fig. 2. Westergren–HumaSed and Westergren–ESR-Auto Plus® Bland–Altman (difference) plots. The open circles are the data points, the solid black lines are the reference lines, the solid blue lines represent bias, and the dashed lines are 95% limits of agreement. Note the large bias in the Westergren–ESR-Auto Plus compared with the HumaSed–Westergren comparisons. ESR, erythrocyte sedimentation rate.

no effect on the ESR measurement by either of the instruments.

DISCUSSION

Three hundred and seventy-five ESR analyses were performed on samples from 125 patients using the three ESR measurement methods. Samples with missing or incomplete values from any one of the three methods were not included in the analysis. The measurement range for the HumaSed is slightly higher and that of the EAP is slightly lower than that of the Westergren (Table 1). The analytical ranges of the three methods show significant overlap and all encompass the 15–105 mm/hr range specified by the NCCLS guideline (8). The linear range of the EAP is 1–101 mm/hr, which is identical to that of Westergren, whereas that of the HumaSed is slightly higher at 1–121 mm/hr.

The ESR mean values from the two automated methods were slightly higher but the 95% confidence

intervals of the mean across the three methods show overlap (see Table 1). These mean values are therefore comparable and clinically not different. The actual and mean difference values between Westergren and HumaSed and Westergren and EAP were all small with individual differences less than 12 mm/hr. Although the %CVs are high, they are quantitatively very close together indicating very small inaccuracy and systemic bias between the Westergren and the other test methods. In the recent pilot ESR proficiency program of the RCPA, the CVs of the various measurement methods were found to be high. The results obtained from this analysis are comparable to those obtained in the pilot ESR proficiency programs (19).

The high correlation coefficients between 0.91 and 0.96 (given in Table 2) indicate strong association between the ESR values obtained by the Westergren and each of the automated methods. This association is

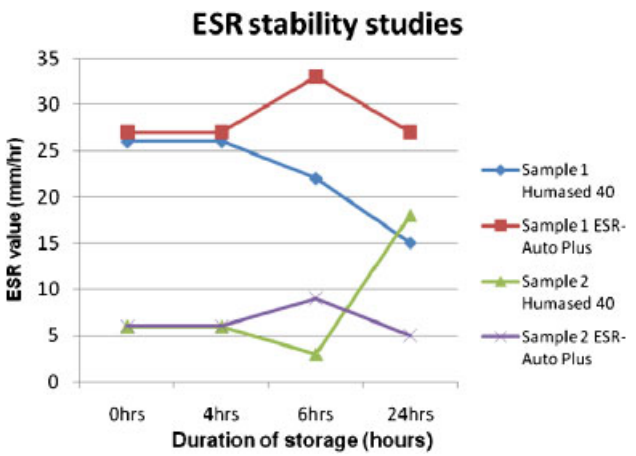

Fig. 3. ESR stability study results of the various analyzers. ESR, erythrocyte sedimentation rate.

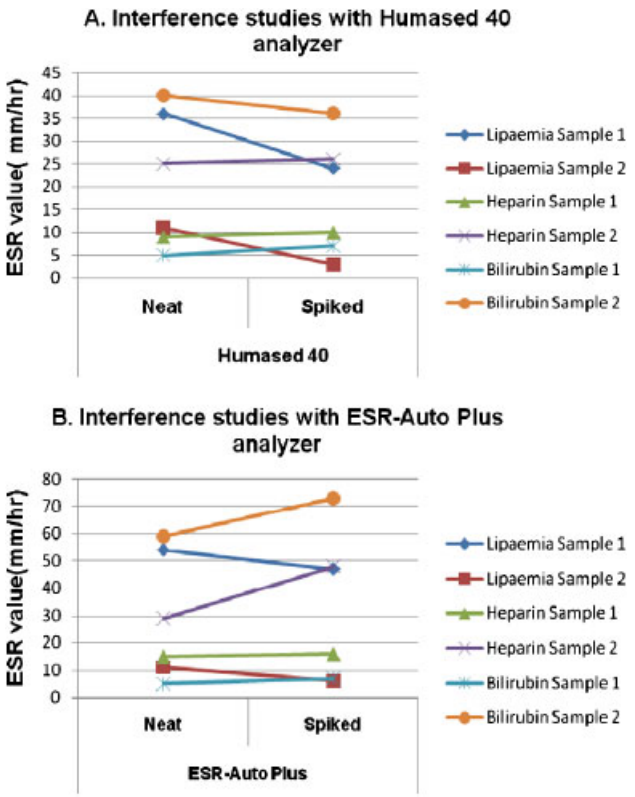

Fig. 4. Interference studies with HumaSed 40 and ESR-Auto Plus® analyzer. ESR, erythrocyte sedimentation rate.

TABLE 4. Summary Precision Data

	Level 1 ESR control		Level 2 ESR control	
	HumaSed	ESR-Auto Plus®	HumaSed	ESR-Auto Plus®
A. Within-run precision of controls over ten runs				
Measured mean	9.91	8.60	58.90	64.00
SD	1.64	0.97	2.18	2.67
%CV	10.23	11.23	3.71	4.17
Expected mean	14.00	10.00	56.00	61.00
Expected range	5–15	3–17	48–72	43–79
%Bias	−29.21	−14	5.17	4.92
B. Between-run precision of controls over ten days				
Measured mean	8.10	7.50	58.40	64.20
SD	1.20	1.08	3.31	3.26
%CV	14.78	14.40	5.66	5.06
Expected mean	14.00	10.00	56.00	61.00
Expected range	5–15	3–17	48–72	43–79
%Bias	−42.12	−25	3.57	5.24

ESR, erythrocyte sedimentation rate; SD, standard deviation; %CV, percent coefficient of variation.

stronger between the Westergren and EAP ESR values than it is between the Westergren and HumaSed values. The HumaSed–EAP ESR association lies between these two extremes. These small differences are clinically not significant.

The ordinate intercepts and their corresponding 95% confidence interval values given in Table 2 indicate difference of a constant nature between the three methods. Slightly higher values were obtained with the machines compared with the Westergren manual method. Although statistically significant ($P = 0.0001$), these differences in ESR values were all less than 12 mm/hr and were within the published allowable ESR limits (2). They are clinically not significant.

The 95% confidence interval for the slope for the Westergren/HumaSed comparison is 0.848–0.978 and does not encompass the ESR value of 1 (Table 2). This indicates proportionate differences between the two methods, with the HumaSed overestimating the ESR value. All three slope comparison results show statistically significant differences across all methods; however, these differences are clinically not significant.

In the comparative scatter plots shown in Figure 1, most ESR values are outside the 95% confidence interval but within the 95% prediction interval. There are very few outliers in these scattering plots and notable absence of systemic bias between the methods compared in these scatter plots.

The HumaSed difference scatter plot shows a bias of 0.6, which is ten times less than EAP bias of 6.6 (Fig. 2). However, the limits of agreement between the HumaSed and the Westergren are very wide (−20.2 to 21.3) making this difference in bias clinically not significant.

Both the HumaSed and EAP difference plots (Fig. 2) show that the majority of the data points are within the 95% limits of agreement with a few outliers. The outliers occur in the middle of the data sets, which rules out possible data skewing. They have been verified as true measurements and not owing to clerical errors. The significance of these outliers is uncertain and their presence appears not to affect interpretation of this evaluation data.

Both the HumaSed and EAP analyzers have unacceptable high imprecision for the within-run and between-run analysis of level 1 ESR control (Table 4). As the level 1 control encompasses the ESR of the normal population, this imprecision is clinically not significant. In contrast, the level 2 ESR control imprecision is low at approximately 5%. This low level 2 imprecision encompasses the clinically significant ESR range.

There is no difference in the ESR controls 1 and 2 imprecision between the two automated analyzers both in the within-run and between-run analyses.

The stability studies confirmed that samples for ESR analysis using either the HumaSed or the EAP are not stable beyond 4 hr (Fig. 3). All ESR analyses should be performed within 4 hr from the time of collection as per NCCLS and ICSH recommendations.

In this interference analysis, heparin appears to have no effect on the ESR measurement by either the HumaSed or EAP methods. Bilirubin shows no interference with ESR measurement at low or normal values. High ESR values are unreliable in the presence of bilirubin for both automated analyzers. The presence of lipids in blood results in the underestimation of the ESR values by both the HumaSed and EAP analyzers.

Compared with the Westergren, the two analyzers were simple to set up and required minimal instruction for consistent performance. The total analytical time per sample was reduced in the automated methods compared with the Westergren. We experienced no down time of either of the two autoanalyzers during the 5-day evaluation period in this study.

There are currently no published studies in the English literature comparing either of the two semi-automated methods with the Westergren. Our study is the first to do a three-way comparison of these methods.

In conclusion, in this three-way comparison of ESR measurement methods, accuracy and precision of the two semi-automated methods were found to be comparable to the Westergren. The semi-automated methods offer a number of potential advantages over the Westergren method and may be used interchangeable with the Westergren in the modern clinical laboratory.

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REFERENCES

1. ICSH. ICSH reference method for the erythrocyte sedimentation rate (ESR) test on human blood. Scand J Haematol 1973;10:319–320.
2. ICSH. ICSH recommendations for measurement of erythrocyte sedimentation rate. International Council for Standardization in Haematology (Expert Panel on Blood Rheology). J Clin Pathol 1993;46:198–203.
3. Zlonis M. The mystique of the erythrocyte sedimentation rate. A reappraisal of one of the oldest laboratory tests still in use. Clin Lab Med 1993;13:787–800.

4. Zweegman S, Makkink B, Stehouwer CD. Giant-cell arteritis with normal erythrocyte sedimentation rate: Case report and review of the literature. Neth J Med 1993;42:128–131.
5. Brigden ML. Clinical utility of the erythrocyte sedimentation rate. Am Fam Physician 1999;60:1443–1450.
6. Larsson A, Hansson LO. Inflammatory activity: Capillary electrophoresis provides more information than erythrocyte sedimentation rate. Ups J Med Sci 2005;110:151–158.
7. Happe MR, Haparano DF, Dooley DP, et al. Validation of the Diesse Mini-Ves erythrocyte sedimentation rate (ESR) analyzer using the Westergren ESR method in patients with systemic inflammatory conditions. Am J Clin Pathol 2002;118: 14–17.
8. Koepke JA, Simson BB, van Assendeft E. NCCLS reference and selected procedure for the erythrocyte sedimentation rate test—approved standard. NCCLS document H2-A4, Vol. 20(27)(H2-A4), 2000. p 1–24.
9. Besson I, et al. Evaluation of 3 automatic systems for measurement of the erythrocyte sedimentation rate. Sangre (Barc) 1995;40:103–107.
10. Tabuchi T, Tominaga H, Tatsumi N. Problems related to rapid methods for erythrocyte sedimentation rate test and their solution. Southeast Asian J Trop Med Public Health 2002; 33:151–154.
11. Piva E, Fassina P, Plebani M. Determination of the length of sedimentation reaction (erythrocyte sedimentation rate) in non-anticoagulated blood with the Microtest 1. Clin Chem Lab Med 2002;40:713–717.
12. Horsti J. EDTA samples for the measurement of erythrocyte sedimentation rate using Sedimatic 100 analyser. Clin Chem Lab Med 2001;39:1001–1003.
13. Kallner A. On the temporal development of erythrocyte sedimentation rate using sealed vacuum tubes. Am J Hematol 1991;37: 186–189.
14. Plebani M. Erythrocyte sedimentation rate: Innovative techniques for an obsolete test? Clin Chem Lab Med 2003;41:115–116.
15. Human. 2006. Humased 40 Operator's Manual. Wiesbaden, Germany: Humased.
16. Streck. 2005. ESR-Auto Plus Operator's Manual. Leeds, UK: Streck.
17. Krouwer JS, et al. NCCLS method comparison and bias estimation using patient samples; approved guideline—second edition, document EP9-A2, Vol. 22(19(EP9-A2)). NCCLS, 2002. p 1–55.
18. Aulesa C. Validation of the Coulter LH 750 in a hospital reference laboratory. Lab Hematol 2003;9:15–28.
19. RCPA. RCPA Haematology Quality Assurance Program (web site), 2006 [cited March 2008]. Available from: http://www.rcpaqap.com.au/haematology/annualreports/uploadedfiles/annualreport2006.pdf.