30 MINUTE VALUES

ESRs are now available in 30 minutes on the Starrsed analyser. Richard Rogers from the Royal Berkshire Hospital demonstrates how.

Whilst largely devoid of specificity, the Erythrocyte Sedimentation Rate .ESR) is a frequently requested test in many laboratories. It is used as a general screen of ill-health, (particularly for inflammation and malignancy), and as an index of the progress of disease in individual patients after a diagnosis has been made. The test is cheap, quick and can be performed by staff with relatively little training.

Strictly speaking, the ESR is not a measurement of the rate of sedimentation of erythrocytes through autologous plasma since the degree of sedimentation is not linearly related to time over the total duration of the test. More correctly, the ESR is simply a measurement of the distance in millimetres that the erythrocytes have settled in the first hour of sedimentation, hence the units in which the result is conventionally expressed, i.e. mm in 1 hour.

The test is an indirect reflection of plasma protein changes that occur in many disease processes. Sedimentation of erythrocytes through plasma occurs in three stages:-

i) Rouleaux formation. Plasma proteins potentiate rouleaux formation, in which erythrocytes aggregate in a specific manner, resembling a stack of coins.

ii) Sedimentation. Erythrocytes aggregated into rouleaux sediment faster than single cells. During sedimentation, a counter-current of plasma is set up as falling erythrocytes displace fluid upwards, which retards the process.

iii) Packing. Under conditions where there is both a high ESR and/or a high haematocrit, erythrocytes pack in the bottom of a sealed sedimentation tube, ultimately approximating to the packed cell volume. The rate of sedimentation decreases as packing retards the downward movement of erythrocytes.

The Starrsed performs ESRs in accordance with the ICSH [1] reference method. The analyser aspirates approximately 1.7 ml. of EDTA or pre-citrated blood by means of the closed-vial sampler from an evacuated specimen container. (An autosampler is soon to be introduced). An accurately metered quantity of tri-sodium citrate diluent can be added automatically to EDTA blood if required, as the sample is withdrawn. Blood is automatically drawn up to a column height of 200 mm (+/- 10 mm) in one of 120 Westergren glass sedimentation tubes, mounted in a rotating carousel. As the aspirate cycle for one sample finishes, the carousel rotates to allow another tube to be filled from the next sample. Later, the tubes are presented at the appropriate time to the infra-red reading device. This scans the filled Westergren tubes in turn to measure the overall height of the blood column and the distance from the meniscus to the plasma-erythrocyte interface, which is reported by the analyser as the sedimentation reading. The tubes are then automatically washed and dried ready for re-use. The sedimentation time is user-selectable, although measurements taken at times other than 60 minutes are not directly comparable with those obtained under ICSH recommended conditions.

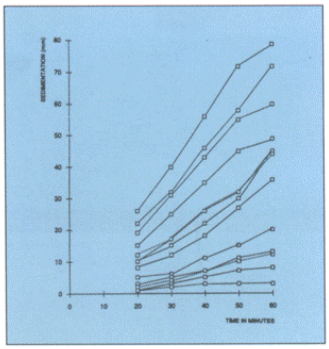

Figure.1 Sedimentation v time

The Starrsed has previously been shown to produce results which compare well with those produced by an ICSH selected (Westergren) method [2,3]. It offers considerable benefits in consumables savings, (the glass sedimentation tubes are automatically washed and dried ready for re-use), health and safety (closed vial sampling, with no contaminated consumables for disposal) and workflow (sedimentations are read automatically, the results being available for transmission to a laboratory computer system).

The aim of this investigation was firstly to investigate the relationship on the Starrsed between the "traditional" 60 minutes ESR and a sedimentation measurement made after a shorter period of time, and secondly, to establish whether it is possible to use this relationship to predict valid results comparable with those produced by the reference method, from a shorter sedimentation period. Significant shortening of the sedimentation time would allow a better service to be provided in terms of result turnround time, and would enable significant workflow improvements to be made in our laboratory.

All of the samples used in this investigation were taken into EDTA, the Starrsed being used in the automatic citrate addition mode.

Choice of sedimentation time

Figure 1 shows the results of a small-scale^^ study involving 13 different samples with a range I of ESR results from normal to significantly elevated. Dotted lines arc used to clarify line crossovers or merges. The samples were tested manually using citrated blood and Westergren pattern disposable plastic sedimentation tubes.

In choosing a sedimentation time shorter than the ISCH standard, the time clearly has to represent a significant saving whilst avoiding poor agreement with 60 minute results. In addition, it was necessary to ensure that even in very low ESRs, there is adequate sedimentation for the Starrsed reader, which has a resolution limit of approximately 0.25 mm, to detect a plasma: cell interface.

From the results shown in figure 1 it was decided to choose 30 minutes as the sedimentation time for the basis of the study on the Starrsed, since this time is short enough to give useful improvements in workflow, (doubling the throughput capacity of the analyser) whilst being adequately discriminating between significantly different 60 minute values. It has also been shown by Berzelius *et al*. |4| that the correlation with 60 minutes sedimentations is significantly inferior if times less than 30 minutes are used.

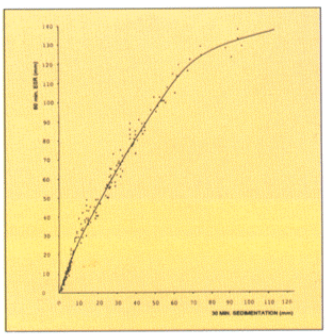

Figure.2 Scatter plot – 30 min sedimentation v 60 min ESR

Investigation of the relationship between 30 and 60 minute sedimentation values

With the Starrsed set to time for 60 minutes, 214 fresh samples (i.e. taken and tested on the same day) were tested on the analyser over a period of time. They were read manually after 30 minutes whilst on the Starrsed using the specially adapted ruler supplied with the instrument, and then read again, automatically, at 60 minutes (having previously verified the comparability of manually and automatically read sedimentations). The 30 minute sedimentation measurements were then plotted against the 60 minute ESR values. Samples showing indeterminate interfaces such that manual reading would have been inaccurate

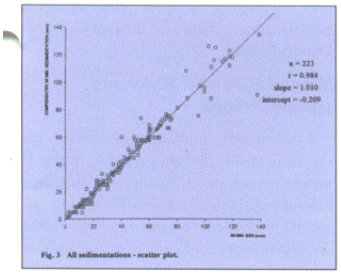

Figure 3 All sedimentations – scatter plot

were ignored. The samples used were selected to provide as wide a spread of results as possible. The results of this comparison are shown in figure 2. From the graph in figure 2, 60-minute equivalent ESR values corresponding to sequential 30 minute sedimentation readings were read off to ^produce a compensation table, which was used in this evaluation to predict 60 minute-equivalent ESR results from 30 minute sedimentation readings made by the Starrsed.

Verification of the 30 minute sedimentation - 60 minute ESR relationship

Strictly speaking, figure 2 is not a comparison of like with like since the 30 minute measurements were made manually whilst those at 60 minutes were read automatically by the Starrsed. The graph was derived this way because it allowed the pairs of readings to be made as close together in time as possible, with the objective of minimising any bias that might arise as a result of sample ageing. To verify that the established relationship was applicable to 30 minute readings made by the Starrsed, and that the derivation of the compensation table from figure 2 was accurate, a few fresh samples per day (amounting 223 in total) were tested twice on the machine, once with the sedimentation time set to 60 minutes,

and then with a setting of 30 minutes. The analyses were performed as close together as the practicalities of the routine work allowed (i.e. the last few samples in the day were re-tested on a 30 minute setting after the end of the day's routine work) to minimize changes due to sample ageing. The results of this comparison are shown in the scatter plot, Fig. 3.
The correlation coefficient relating predicted to measured Starrsed ESR values was 0.984 using the least squares method of linear regression analysis. Berzelius et al. [4] obtained an almost identical figure of 0.982 using 30 minute sedimentations in 100 mm collection/sedimentation tubes (Becton Dickinson and others), read on a modified Sedimatic 100 ESR machine (Analys Instrument, Sweden).

The assessment of agreement between two methods

The correlation coefficient is actually a measurement of the strength of a relationship between two variables and not of the agreement between them, despite being frequently used in method comparisons. In comparisons of two methods for the same measurement, it would be rather surprising if the results were not related. An r value of 1.0 would be obtained if all of the points fitted on to any straight line, even if one method gave results only 10% of the value of those obtained by the other. In figure 3, the slope of 1.010 and intercept of -0.209 (which are not significantly different from the ideal values of 1.0 and 0 respectively), and correlation coefficient of 0.984 indicate that the results of the two ESR methods compared here show excellent agreement over the range of values obtained, and that there is essentially no overall bias. However, significant

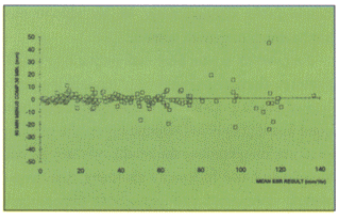

Figure 4 All sedimentations – difference plot. (n=223)

local areas of bias that cancel each other out over the entire range may exist. A better method for assessing the agreement between two methods and demonstrating bias is the difference plot as described by Altman and Bland [3L in which the mean of the two results is plotted against the difference between them. Figure 4 shows a difference plot of the same data as that shown in figure 3.
The difference plot suggests that the relationship between 60 minute ESRs and compensated

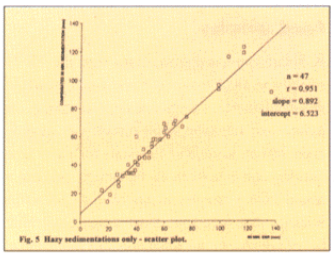

Fig.5 Hazy sedimentations only - scatter plot

30 minute sedimentation readings is free of significant local and overall bias, i.e. the points are evenly distributed either side of the zero difference line throughout the range of measurements. The degree of bias can be calculated:-

Sum of (60 min ESR minus compensated 30 min sedimentation) = -69
number of values = 223
Therefore mean difference (bias) per result = -0.3 mm in 1 hour

i.e. for mean Starrsed sedimentation values in the range 0-140 mm in 1 hour, the compensated 30 minute sedimentation values are, on average, 0.31 mm in 1 hour greater than the 60 minute ESRs. This corresponds approximately with the 1% slope error of 1.01, and represents insignificant bias in the case of ESR results.

Hazy readings

The Starrsed designates sedimentations as 'ha/y when the plasma-erythrocyte interface is diffuse over a pre-defined distance rather than being a clear demarcation. Since it would seem possible that ha/y 30 minute sedimentations might give rise to erroneous ESR results, these were examined as a separate group. The scatter and difference plots for this group are shown in figures 5 and 6 respectively.
It is to be expected that the correlation of these sedimentations alone would be less good than those with a more distinct plasma-erythrocyte interface. However, it should be borne in mind that manual reading of hazy sedimentations is much more prone to imprecision that automatic reading on the Starrsed, which recognizes the top of the erythrocyte column by a pre-determined increase in infra-red opacity as the Westergren tube is scanned by the reading device. 'I he mean difference of-1.0 for ha/y sedimentations shows that the compensated 30 minute sedimentation obtained from figure 2 represents an overestimate relative to the 60 minute ESR of 1mm in 1 hr. The nature of the ESR measurement is such that in probably every case, none of the differences shown in figures 5 and 6 arc of clinical significance.

Aged samples
ICSH recommendations state that the EDTA sample should be tested within 4 hours of venepuncture(l). The practicalities of sample collection and transport from distant GP surgeries make this an unrealistic timescale for many laboratories, and it appears to be general practice to regard EDTA samples as suitable for ESR testing if refrigerated overnight. Since a significant number of samples in our laboratory fall into this category, it is necessary to determine whether the relationship shown in figure 2 for fresh samples allows the conversion of 30 minute sedimentations from older samples into valid 60 minute ESR results. All of the samples used were at least 24 hours old.
These results are shown in Figures 7 and 8. It can be seen from fig. 8 that aged samples show obvious bias, corresponding to an average overestimation of ESR from 30 minute sedimentation values relative to 60 minute measurements

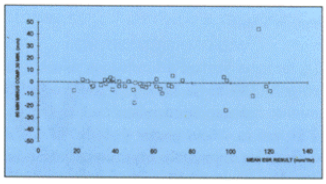

Fig.6 Hazy sedimentations only - difference plot (n=47) mean difference = -1.0

Discussion
Since ESR results are indicative in nature rather than being precise and accurate measurements of a specific analyte, it is felt that the differences between results obtained by testing under ICSH conditions and from compensated measurements taken after a shorter sedimentation time are acceptable and clinically insignificant. To put these differences into perspective, in another (unpublished) investigation performed in our laboratory, we have found that by comparison with EDTA samples run on the Starrsed in the automatic citrate addition mode, samples taken into commercially produced, pre-citrated bottles frequently produce errors in the ESR value of 30% and occasionally in excess of 50%, apparently due to inaccurate filling with blood during specimen collection. It has been shown by Berzelius *et al* (4) that reading during the sedimentation phase (i.e. around 30 minutes) of very high ESRs may actually give a better reflection of the true result than a reading taken at 60 minutes,

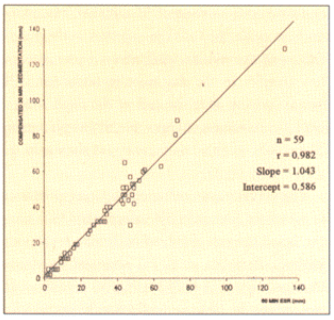

Fig.7 Scatter plot - 24 hour old samples

when erythrocyte packing causes the sedimentation rate to decrease. 3% of inpatient samples were found to fall into this category.
The findings of our investigation using the Starrsed show that the machine is capable of generating 30 minutes sedimentation results which can be used to predict valid, 60 minute-equivalent ESR results. The relationship demonstrated in this investigation provides a very useful way of speeding up ESR throughput in our laboratory, primarily because a result is available much more quickly. There is an additional, important advantage related to the fact that it is impossible to aspirate more samples for ESR if in so doing it would be necessary for the Starrsed to rotate unread sedimentations past the reading sensor. Due to the increased throughput capacity that 30 minute sedimentation allows in comparison with 60 minutes, this situation arises very rarely, and never causes a significant delay in our laboratory. This is an important improvement, since depending on the size of the ESR batch, the delay when using 60 minute sedimentation was frequently considerable. Consequently, we are able to provide a much improved service.
Even when set to time sedimentations for 60 minutes, the Starrsed obviously resolves the problem of reading ESRs after laboratory staff have gone home. However, depending on the working practices in operation, the results may not be validated until the following morning, and hence remain unreported and unavailable from outside the laboratory. Using 60 minute sedimentations in our laboratory, (which receives a large quantity of late work, especially from GPs), 30-60 reports usually missed the last report collection whilst awaiting ESR results. By changing the sedimentation time to 30 minutes and programming the laboratory computer to perform the compensation automatically, this figure has been reduced to around 10-20. Additionally, the laboratory is able to produce reliable ESR results 24 hours a day to rival the speed of those obtained by local near-patient testing (taking transportation times into account), in those cases where the test is of significant diagnostic importance.

Conclusions

* The prediction of 60 minute-equivalent ESR results from Starrsed 30 minute sedimentation readings give results which agree will with 60 minute ESR results. The differences between the methods do not appear to be of clinical significance.
* The relationship established in this investigation is valid for 24 hour old samples as well as those taken and tested on the same day. 30 minute sedimentations that the Starrsed flags as hazy can be confidently used to predict valid 60 minute - equivalent ESR results.
* Timing sedimentations for 30 minutes effectively more than doubles the throughput capacity of the analyser. This has allowed a significant improvement in report turnround time in our laboratory, resulting in the provision of an. improved service.

Manual ESRs and 30 minute sedimentations
A continuation of this investigation shows that the relationship demonstrated between 30 minute sedimentation readings and 60 minute ESRs also applies to the manual Westergren-type method. It should be pointed out, however, that the validity of the conversion would be markedly reduced due to the amplification of potentially serious reading and correction errors, especially where the plasma - cell interface is hazy.

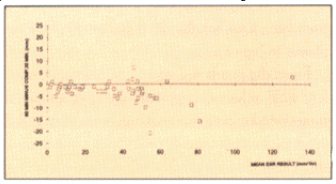

Fig.8 Difference plot - 24 hour old samples

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