Technical methods

A micromethod for the erythrocyte sedimentation rate suitable for use on venous or capillary blood

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The erythrocyte sedimentation rate (ESR) is a useful non-specific test applied to screening for, or the serial monitoring of, disease states. The Westergren ESR, as measured by the reference method of the International Committee for Standardisation in Haematology,[1] requires at least 1 ml of blood. Methods using smaller volumes, including capillary samples, have been described,[2-4] but, with the exception of the Accu-tech method described by Stuart *et al.*,[3] for which the tubes are not commercially available, these methods utilise glass capillary tubes, which are susceptible to leakage and breakage.

Polystyrene Westergren ESR tubes are now available and have been shown to give results comparable to that of the reference Westergren method.[5 6] The micro ESR method described utilises polystyrene tubes, and a comparison with the reference Westergren method is reported.

Clinical materials and methods

Specimens were taken into 1·8% w/v dipotassium ethylene diamine tetra acetic acid (EDTA). Capillary blood samples from 40 children were collected as previously described,[3] and 18 of these children agreed to have duplicate capillary samples taken for assessment of sampling error. Venous blood samples were taken, using a 21 gauge butterfly needle, from the same 40 children approximately 90 minutes later, before an intravenous infusion of cytotoxic drugs. Venous blood samples sent to the department for routine analysis from 200 adults and children were used to compare the micro ESR method with the reference Westergren method.

All samples were tested within 3 hours of receipt in the laboratory.

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METHODS

Capillary blood samples were tested by the micro method only and venous samples were tested by both methods.

*Micro ESR (Guest Dispette)**

EDTA blood was added to 31·3 g/l trisodium citrate (0·2 ml blood; 0·05 ml citrate) in the funnelled reservoir cap and mixed, care being taken to avoid the formation of air bubbles. Plastic disposable micro ESR tubes, 230 mm long with an internal bore of 1 mm and graduated to 150 mm, were specially prepared by the manufacturers. Blood enters the ESR tube by means of the positive pressure created by the insertion of the tube into the cap. The height of the blood column is adjusted to zero by manipulating the ESR tube within the cap. The tube and cap were maintained in a vertical position by the Dispette tube holder and were stood at room temperature for 1 hour before the ESR was read.

Westergren ESR

This was measured according to the reference method[1] using Westergren-Katz glass ESR tubes.[7]

Statistical significance of the results was determined by the Wilcoxon signed-rank test for matched pairs and by the F distribution for difference between variances.

Results

ASSESSMENT OF REPRODUCIBILITY OF MICRO ESR METHOD

Duplicate micro ESR determinations were performed on 53 venous blood samples with ESR values ranging between 1 and 120 mm/hour. The results showed good correlation ($r = 0{\cdot}99$), as shown in Fig. 1, with no significant difference ($P > 0{\cdot}4$) between the paired results. No significant difference ($P > 0{\cdot}1$) was demonstrated between the micro ESR results on the 18 duplicate capillary samples.

COMPARISON BETWEEN MICRO ESR AND WESTERGREN ESR USING VENOUS BLOOD

Venous blood samples were used to compare the

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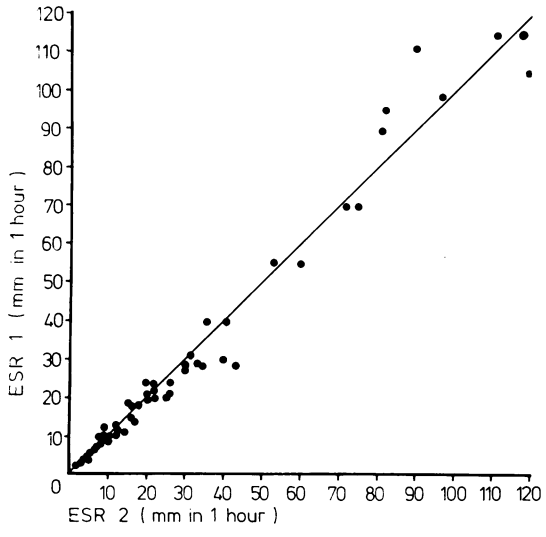

Fig. 1 *Duplicate micro ESR values from 53 venous blood samples.*

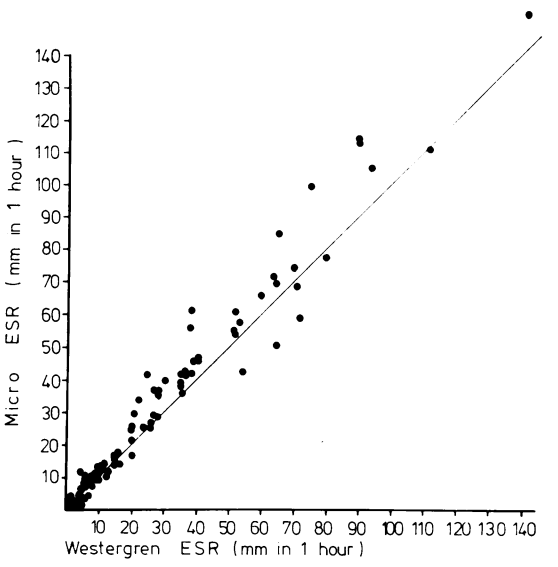

Fig. 2 *Comparison between micro ESR and Westergren ESR on venous blood from 107 patients.*

micro ESR method and the reference Westergren method. The correlation between the ESR results was good, as shown in Fig. 2, but the individual paired values showed the micro ESR result to be significantly higher ($P < 0.01$).

COMPARISON BETWEEN MICRO ESR USING CAPILLARY BLOOD AND WESTERGREN ESR USING VENOUS BLOOD

Micro ESRs were set up using capillary blood from 40 children and compared with the reference Westergren ESR as determined using their own venous blood. The ESR values obtained by the two methods correlated well ($r = 0.94$), showed no significant difference between paired results from the same child ($P > 0.3$), and no longer showed higher values in the micro method.

Discussion

The micro ESR as determined using capillary blood compares favourably with the Westergren method. When venous blood is used, however, the micro ESR appears significantly higher. This phenomenon could be accounted for by the haematocrit difference between venous and capillary samples.[8] Closer examination of the comparison between these results using venous blood shows that the difference between the paired values was most marked with ESR levels greater than 60 mm/hour. The difference between the two methods was negligible at ESR levels up to 40 mm/hour.

Methods currently available for micro ESR determination give poor correlation with the reference Westergren method. The Accu-tech micro method, as described by Stuart *et al.*,[3] was reported to give good results but the ESR tubes are not commercially available.

The micro method described is simple to perform and reproducible and requires only 0·2 ml blood. It has been shown in this study to correlate well with the reference Westergren method. The method can be applied to either venous or capillary blood and is a completely disposable system allowing safe handling of samples. The method could be a potentially useful substitute for the Westergren method, particularly in paediatric laboratories using mainly capillary samples, or when only small volumes of blood are available for examination in a fully automated department.

We are grateful to Guest (Medical and Dental) Limited for supplying the micro ESR tubes, and to Professor J Stuart and Dr FGH Hill for their advice during the preparation of this paper.

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Saving tests by pooling sera—how great are the benefits?

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When a large proportion of a population is negative for a serological factor, it may be possible to reduce the number of tests needed to identify positive individuals by first testing pooled samples and then re-testing individually the sera from any pool that gives a positive result.

If the proportion of negatives in the population is known, the pool size that will minimise the average number of tests needed to get a result for each of a set of sera can be readily established. Suppose sera from N individuals, each with probability p of being negative, are pooled in groups of size k, where k is a factor of N. If all the individuals contributing to a pool are negative, only one test will be used. The probability of this happening is p^k. If, however, the result from a pool is positive, an event occurring with probability $1 - p^k$, further k tests will be needed for that pool. Thus the status of the k individuals contributing to a pool will be determined by either 1 test, with probability p^k or $k + 1$ tests, with probability $1 - p^k$. As N/k is the number of pools, the expected number of tests needed to establish the status of the N individuals is

$$N/k \times (1 \times p^k + (k + 1) \times (1 - p^k))$$

which equals

$$(N/k) \times (1 + k\,(1 - p^k)) \quad \ldots.(A)$$

If k is not a factor of N, there will be one or more sera, r say, left after Int (N/k)* pools of size k have been made. If these are treated as a separate pool of size r, the average number of tests needed to examine N sera will be

$$\text{Int}\,(N/k) \times (1 + k\,(1 - p^k)) + 1 \times (1 + r\,(1 - p^r)) \quad \ldots.(B)$$

Alternatively, each of the remaining r sera might be added to one of the pools of size k, which would make the average number of tests required

$$\text{Int}\,(N/k) - r) \times (1 + k\,(1 - p^k)) + r \times (1 + (k + 1)\,(1 - p^{k+1}) \quad \ldots.(C)$$

As the first practice is probably easier to manage in a laboratory, formula B has been used to calculate the values for Table 1, which shows the average number of tests needed to establish the status of 100 specimens with pool sizes from 2 to 11 for selected values of p, the proportion of negatives in the popu-

*Int (N/k) is the whole number part of the quotient.

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