be directly identified and compared.

The demonstration that immunoreactivity is maintained when tissues are embedded in araldite suggests that further work for its use at the electron-microscope level may now be expected.

References


A rapid and accurate differential centrifugation method for platelet counts

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Various methods have been proposed for preparing platelet suspensions for platelet counting by electronic counters. With some systems the platelets can be counted in the presence of red blood cells, either by the use of hydrodynamic focusing to avoid coincidence and masking of platelets by erythrocytes,1 2 or by computational techniques for estimating platelet counts from extrapolated volume distribution curves.3 Other counting systems require lysis of erythrocytes, leaving intact platelets, which

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are then counted by light scattering or other optical methods. The majority of instruments, however, require the preparation of platelet-rich plasma (PRP); this is obtained either by sedimentation or centrifugation.

Conventional methods of preparation of PRP by centrifugation have been shown to result in a loss of platelets; with some systems the manufacturers recommend methods of centrifuging whole blood, diluted in various fluids, such as 1% dextran, but these, too, have been shown to produce erroneous results. \(^4\) Enhanced sedimentation, as employed by the Coulter Thrombofuge/Thrombocounter system, has been found, in general, to be a reliable and reproducible method;\(^5\) however, it requires PCV correction factors\(^6\) and these are unreliable when the PCV is > 0.5.

Differential centrifugation methods, in which whole blood is diluted in a suspending medium of an appropriate density, have also been shown to provide reliable platelet counts over a wide range of haematological conditions\(^9\)\(^10\) and without the necessity for PCV correction. However, these methods are relatively tedious and time-consuming so that they are unsuitable for use in a routine laboratory.

In this paper we describe a modified differential centrifugation method which allows the platelet count to be carried out easily and rapidly, without loss of accuracy or precision, making it suitable for routine use.

Material and methods

**Preparation of centrifugation fluid**

1. Two ampoules (60 ml) of 32.8% sodium metrizoate solution SG 1.200 ± 0.001 (Nyegaard, Oslo) were poured into a clean, dry 150 ml glass bottle.
2. To this was added 60 ml of Isoton II (Coulter Electronics) and mixed thoroughly for 10 min on a roller mixer.
3. The fluid was filtered through a 0.22 μm micropore filter and stored at 4°C in the dark.

Filtered fluid (10 ml) was poured into a clean, plastic universal container (Sterilin). To this was added 0.1 ml of well mixed, fresh K₂ EDTA (1.5 mg/ml) blood, using a clean, dry pipette. The blood was mixed with the fluid for 30 s by rolling the universal container in the palms of the hands. The suspension was spun in a Mistral M Minor centrifuge (MSE) for 3 min at 2000 g and 8 ml of the red-cell free supernatant removed, poured into a clean Coulter acuvette and remixed for 30 s. A portion (0.2 ml) of this platelet rich suspension was diluted into 10 ml of Isoton II (final dilution 1/5151) and resuspended.

The platelet dilution was counted on a Coulter ZBI/Channelyzer C-1000 link-up using the following settings:
- Lower threshold—5, upper threshold—100
- Aperture current—0.177, amplification—1
- Sampling volume—0.1 ml, orifice diameter—70 μm

The raw count was corrected for coincidence using a Coulter coincidence correction chart, and for dilution, to obtain the whole blood platelet count.

In parallel the manual reference method was performed.\(^11\) For this, whole blood was diluted 1/21 with filtered 1% ammonium oxalate solution, charged into an improved Neubauer chamber and counted under phase contrast.

Sixty-five specimens were counted by both methods; the majority of the specimens were taken at random from the routine haematology laboratory at Hammersmith Hospital. They covered a range of platelet counts from 31-635 × 10⁹/l and included various anaemias, polycythaemias and other haematological abnormalities. The specimens were tested, in all cases, less than three hours after collection.

Marked deviations in speed and time of centrifugation showed no alteration in platelet count in replicate tests. Centrifugation at 1500 g and 2500 g for 3 min respectively did not affect the platelet count, likewise spinning for 2 and 7 min at 2000 g also showed no alteration in count. However, as a routine procedure, the optimum speed and time of centrifugation to obtain a red-cell free supernatant was found to be 2000 g for 3 min.

Results

The Figure shows the plot of the manual reference method versus the proposed centrifugation method. Table 1 shows a comparison between the proposed centrifugation method and weighted Thrombocounter data after analysis of the results from platelet counts on whole horse blood in the UK External Quality Assessment Scheme platelet trials. Table 2 shows the results obtained after platelet counts on polycythaemic and leukaemic blood and on blood with microcytic red cells and microplatelets using both the reference method and centrifugation method.

Comment

By statistical analysis of the results the proposed centrifugation method shows excellent correlation and reproducibility with the manual reference method. A paired t test indicates that the population means of the two methods are not significantly different. In addition, results obtained from platelet counts on whole horse blood by this method in the
Technical methods

EQA Scheme platelet trials are in good agreement with weighted Thrombocounter data (Table 1).

This method is quick, uses small quantities of blood and is relatively cheap by comparison with the Thrombofuge enhanced sedimentation technique. It is reliable for counting platelets in most haematological conditions, including cases where the PCV is greater than 0.5 as the dilutions prior to counting overcome the problem associated with whole blood PRP preparation when the PCV is elevated. With leukaemia samples, the dilution and centrifugation removes interfering white cells, and, as shown in Table 2, good agreement is found between the manual reference and the centrifugation method in these haematologically abnormal situations.

In conclusion, therefore, for any laboratory with a small centrifuge, this method offers a rapid, reliable and alternative way to count platelets without the use of PCV correction factors.

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