«New» Polymorphprep

Polymorphprep was first introduced in 1988, based on a method published by Ferrante & Tong (1980) and the product Mono-poly resolving medium from Flow laboratories.

At that time we decided to use Dextran 500 to aggregate the red blood cells, due to the fact that Dextran 500 was a much cheaper compound than Polysucrose 400 (Ficoll).

During the last few years, there has been an enormous price increase on Dextran 500 whilst the price on Polysucrose 400 has gone down. Therefore, to be able to continue producing this product we had to replace Dextran 500 with Polysucrose 400. Ferrante & Tongs method and the product from Flow laboratories were both based on Polysucrose 400.

A small test batch with Polysucrose 400 instead of Dextran 500 has been produced at Serumwerk in Germany, and the "new" solution has been tested at the John Moores University in Liverpool, UK.

The test results show that the "new" formulation behaves in the same way as the "old" formulation. Therefore, Polymorphprep will be back on the market again in new bottles and caps from end of March.

Test results from Liverpool John Moores University

Protocol

A 15 ml blood sample was taken from a healthy donor into EDTA-coated tubes; 5 ml was loaded on to either 5 ml of Polymorphprep (batch number 12HLS10; expiry date 2017-11) or 5 ml of Polymorphprep Test Batch (Lot L00116) in 15 ml conical polypropylene test tubes. The tubes were centrifuged at 650 g_{av} for 30 min at 20°C. The rotor was allowed to decelerate without the brake (approx. 10 min). After photography of the tubes, the two bands were removed using a flat-tipped metal cannula (i.d 0.8 mm) and examined without any further treatments. The procedure was repeated three times.

Results

The only observable difference from experiment to experiment was the very minor contamination of the lower polymorphonuclear leukocyte (PMN) band with some low density erythrocytes (compare the two images in Fig 1 on page 2) from some blood samples. This is observed with both of the Polymorphprep samples and is routinely removed by washing the cells with isotonic ammonium chloride, which was not carried out in these experiments. All of the observations on the cell harvests were carried out without any washing whatsoever. The resolution of PMNs from PBMCs (peripheral blood mononuclear cells) was unaffected by the presence of erythrocytes.

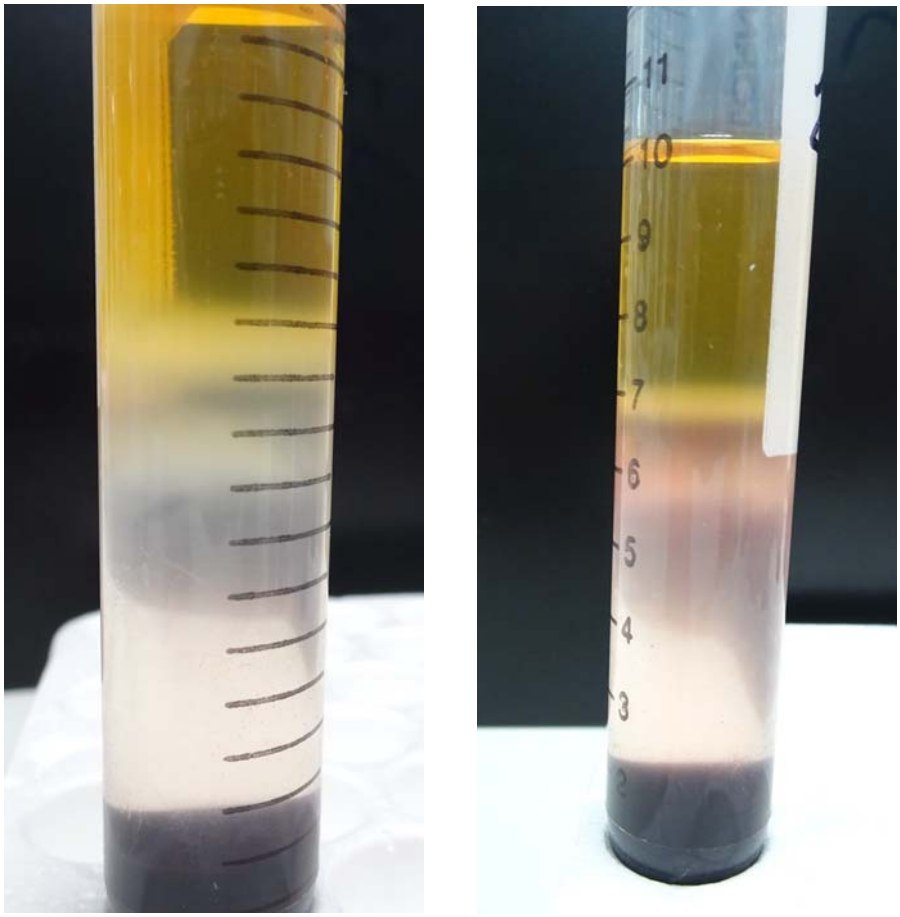

Figure 1. Separation obtained with Polymorphprep Test Batch (Lot L00116) from two separate donations.

The recovered cells were also analyzed by light microscopy after staining. They show that the very minor erythrocyte contamination of the denser band from some donations has no effect on the resolution of the PBMcs and PMNs.

Microscopy

100 µl of cell suspension was fixed for 10 min in 4% paraformaldehyde; the cells were then sedimented onto a poly-L-lysine-coated slide at 500 rpm for 5 min (Shandon Cytofuge IV). Cells were allowed to dry before staining using commercially available reagents (Reastain Quick-Diff)

Staining protocol: (1) fixed 3 x 1 sec in methanol; (2) 16 x 1 sec in eosin; (3) 8 x 1 sec in methylene blue; (4) 3 x 1 sec wash in phosphate buffer. Specimens were air-dried and mounted in DPX.

Three photomicrographs are provided on the following pages.

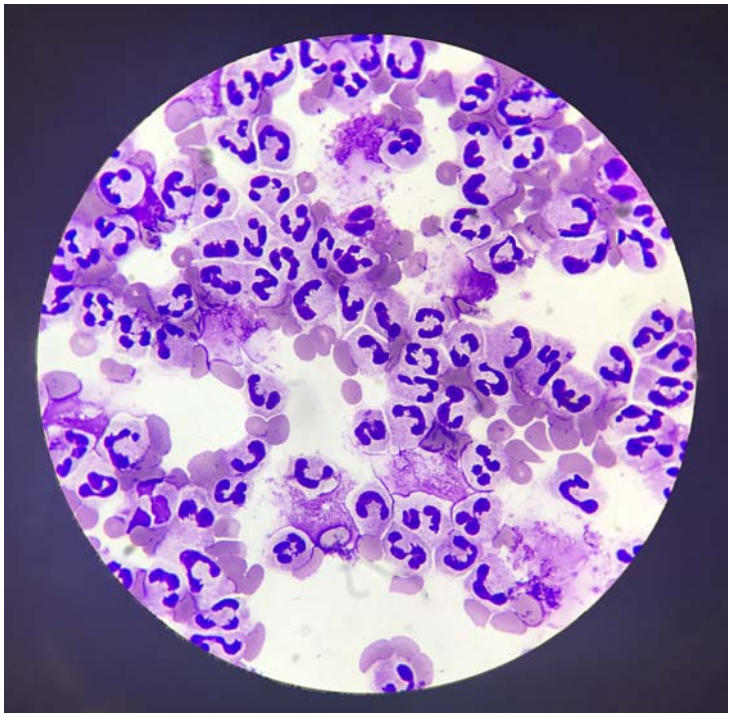

Figure 2 Polymorphprep (batch number 12HLS10; expiry date 2017-11). Lower band.

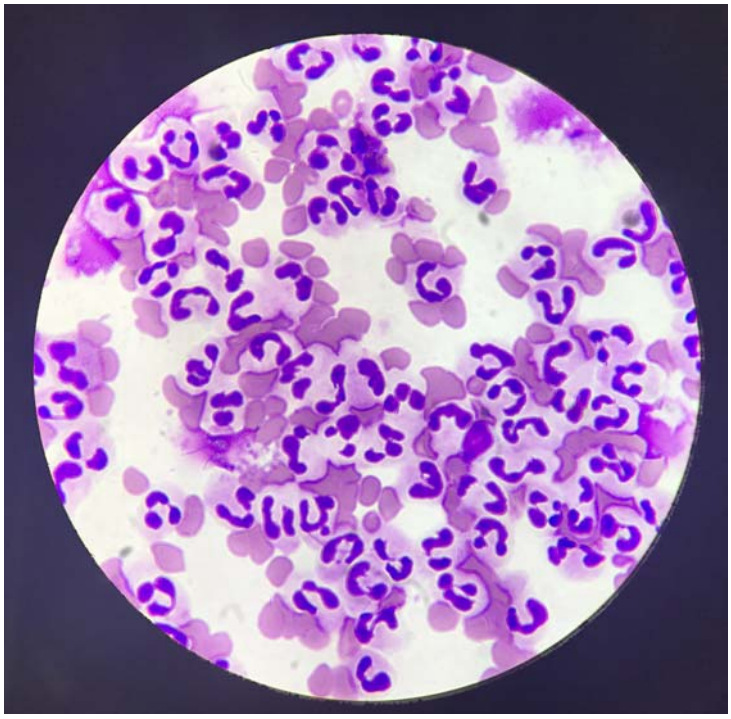

Figure 3 Polymorphprep Test Batch (Lot L00116). Lower band

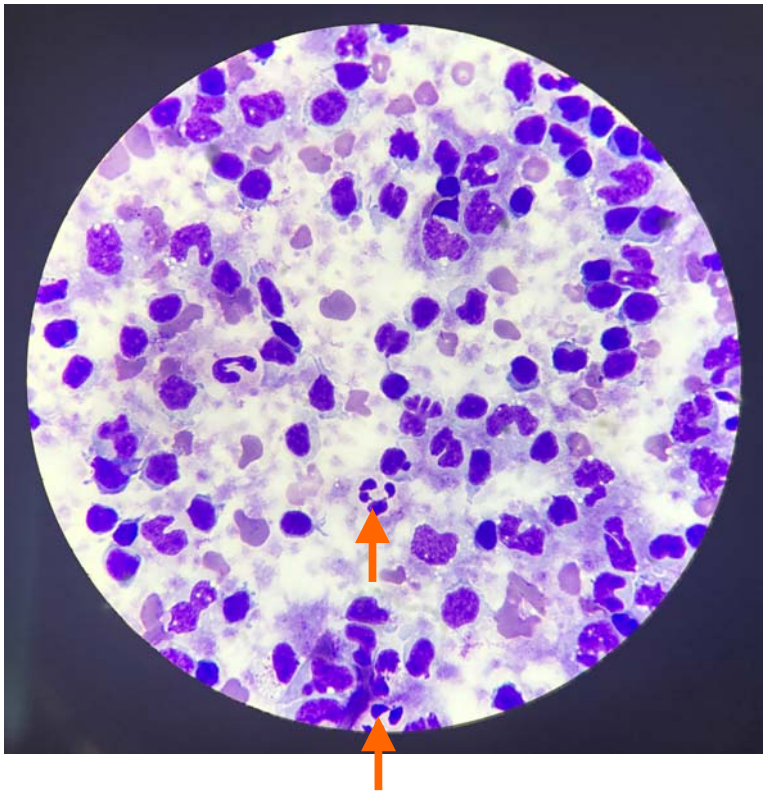

Figure 4 Polymorphprep Test Batch (Lot L00116). Upper band

Figures 2 and 3 show the composition of the lower band. The cell composition in both samples shows the typical nuclei of a PMN. The pink/purple cells showing no nuclei are the contaminating erythrocytes. Compare these images wth that in Figure 4 in which the cells show the typical lymphocyte morphology (cells with a single nucleus occupying almost the entire cell) and monocytes (horse-shoe shaped nuclei). In the figure provided only two PMNs are discernable (shown by arrows). The stippled background is caused by the presence of platelets.

Conclusions

There are no observable differences between the two Polymorphprep samples.