Comparison of Methodologies for the Isolation of Polymorphonuclear Leukocytes from Blood

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ABSTRACT

Grading protocols for the acceptability of wound and sputum specimens for bacteriologic culture are based on the ratio of epithelial cells to polymorphonuclear leukocytes (PMNs) observed in gram-stained smears. To optimize PMN isolation for the preparation of simulated smears used for training by our university-based Clinical Laboratory Science program, three density gradient centrifugation (DGC) methods, Polymorphprep (AXIS-SHIELD, Oslo Norway), Mono-poly (MP Biomedicals (Solon, OH.), Ficoll-hypaque (Sigma, St. Louis, MO) were compared with an immuno-magnetic column-free cell separation method, EasySep (STEMCELL Technologies, Cambridge, MA)). Fresh EDTA blood was collected from one source and used in each extraction according to the manufacturer's recommendations. Cell counts were performed using a Neubauer chamber. Purity of the cells was verified using stained smears. The EasySep was superior to the three DGC methods in ease of performance, PMN concentration (1.5 x 106 cell/ml) and purity (100%). Of the three DGC methods, the Polymorphprep produced the greatest concentration of purified PMNs (6.3 x 105 cells/ml) while the Mono-poly and Ficoll-hypaque methods extracted 1.7 x 105 cells/ml and 2.3 x 104 cell/ml, respectively. For laboratory protocols requiring PMNs such as the preparation of simulated smears, the EasySep method provides an ideal platform for cell separation.

INTRODUCTION

The separation of cellular components from peripheral blood is utilized in a number of research protocols which require the investigation of the properties and interactions of mononuclear cells, as well as polymorphonuclear leukocytes. Until recently, the most common procedure used to separate the different cellular fractions was density gradient centrifugation (DCG), a method first envisioned by Boyum in 1968. Diatrizoic acid coupled with Ficoll, a synthetic polymer of sucrose, is often used to produce a gradient of viscosity during centrifugation to improve separation of the various cellular fractions from whole blood based on their size and mass. A new technique has been developed which utilizes antibody complexes directed to specific cell surface antigens to remove unwanted cells. The antibody complexes link the targeted cells to magnetic particles. The targeted cells are then pulled to the side of the specimen tube when placed in a special magnetic chamber. The cells selected for isolation are poured into a new tube. In this experiment, we compared 3 different DCG methods to the immunomagnetic method for the purification of PMNs from freshly collected EDTA whole blood. The purpose of PMN isolation was the preparation of simulated sputum smears containing inflammatory cells and bacteria for the training of MLS students.

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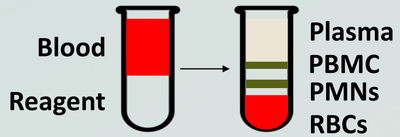
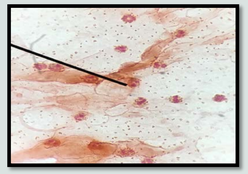
METHODS

Fig. 1.: Neutrophil Isolation by Density Gradient Centrifugation





Sample purity after DGC

Fig 2.: EasySep Neutrophil Isolation

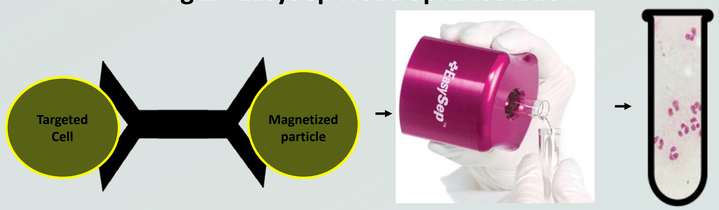


Fig. 3: Stages of EasySep Direct PMN Isolation Procedure

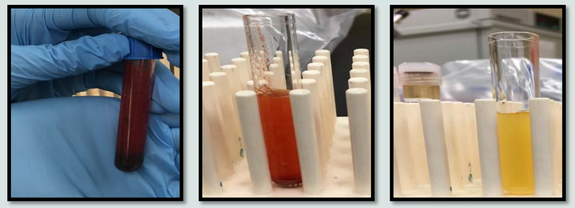
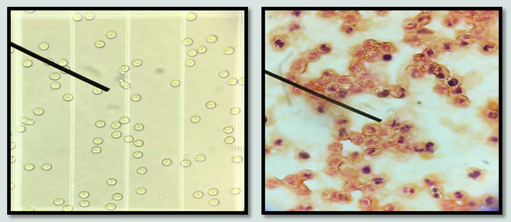


Fig. 4: Sample Purity After EasySep Isolation Procedure



RESULTS

Table 1: Comparison of Reagents and Yields of the Four PMN Isolation Procedures

Method	Isolation Reagent	Time	Yield (cells/ml)
Ficoll- Hypaque Plus	• Ficoll PM400 5.7 g • Sodium diatrizoate 9.0 g • Edetate calcium disodium • Density: 1.077 g/ml	≤ 1.5 hr	2.3×10^4
Mono-Poly	• Ficoll 400 (88.0 mg/ml) • Sodium hypaque 90 (167.6 mg/ml) • Density: 1.114 g/ml	≤ 1 hr	1.7×10^5
Polymorphprep	• Sodium diatrizoate 13.8 % (w/v) • Polysaccharide 8.0% (w/v) • Density: 1.113 g/ml	≤ 1 hr	6.3×10^5
EasySep	• Magnetic Bead/Antibody Complex	≤ 20 min	1.5×10^6

CONCLUSION

Although, the DCG methods provided isolated PMNs, the concentration of retrieved cells was less than the immunomagnetic method. In addition, the preparations were often contaminated with erythrocytes which required an additional lysing step. The immunomagnetic method provided an essentially pure solution of PMNs in a higher concentration and in much less time than the standard DCG protocol. An image of the isolated PMNs in a simulated smear appears below.