Centrifugation and RBC Lysis-Free Preparation of Blood Samples in Under 30 Minutes

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Introduction

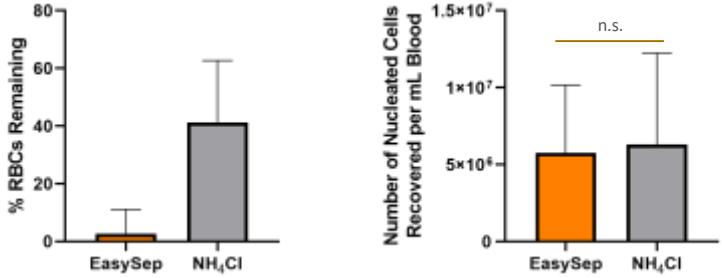
Processing whole blood (WB) to remove red blood cells (RBCs) and/or isolate peripheral blood mononuclear cells (PBMCs) is a common first step in many diagnostic applications. RBCs are typically removed from a blood sample using a RBC lysis agent such as ammonium chloride (NH_4Cl), while PBMCs are prepared using density gradient centrifugation (DGC). Both procedures are time consuming, cumbersome and involve numerous centrifugation steps. We have developed rapid, easy to use immunomagnetic-based methods to either remove RBCs or the combination of RBCs, platelets and granulocytes directly from WB in under 30 minutes. Both procedures were compared against their current gold standard of lysis or DGC, for RBC clearance and PBMC isolation, respectively.

Methods

RBC clearance: WB was either lysed using ammonium chloride (NH_4Cl), or RBCs were removed using the EasySep™ RBC Depletion Reagent .
PBMC isolation: PBMCs were isolated from WB by DGC separation or using the EasySep™ Direct PBMC Isolation kit.
Principal of immunomagnetic separation: Unwanted cells (RBCs and/or platelets and granulocytes) were labeled with antibody complexes and magnetic particles and then placed into a magnet. Labeled cells were retained in the magnet, while untouched nucleated cells or PBMCs were poured into a new tube. Both immunomagnetic procedures can be fully automated on the RoboSep™-S instrument (not shown).

Results

RBC Clearance



PBMC Isolation

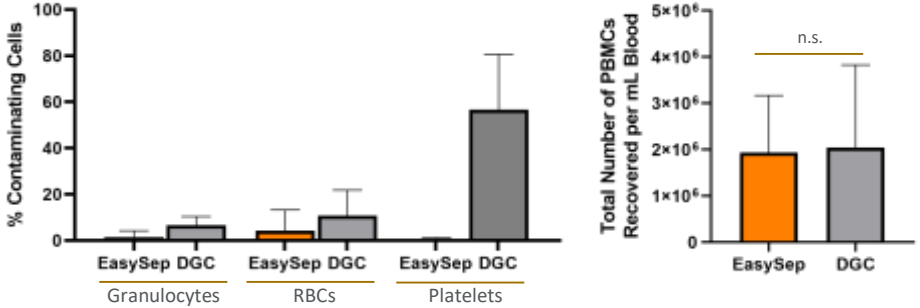


Figure 1. EasySep™ RBC Depletion Reagent provides superior RBC clearance and similar cell yield compared to ammonium chloride lysis (NH_4Cl). Whole blood samples from healthy donors were processed to remove RBCs using either the EasySep™ RBC Depletion Reagent (n=39, all magnet platforms) or NH_4Cl (n=27). Left: After RBC removal, samples were stained with fluorochrome-conjugated anti-CD45 and anti-glycophorin A (CD235a) antibodies and analyzed by flow cytometry. Residual RBCs were identified as glycophorin A+/CD45- events (% of total events, ungated). Right: Total nucleated cells (CD45+) were determined by cell counting and values were normalized to 1 mL of blood. Significance determined using two-tailed, unpaired t-test.

Figure 2. EasySep™ Direct PBMC Isolation gives cleaner PBMC preparations with similar cell recovery compared to density gradient centrifugation (DGC). PBMCs were isolated from healthy donor whole blood samples using either the EasySep™ Direct PBMC Isolation kit (n=30, all magnet platforms) or DGC (n=15). Left: After PBMC isolation, samples were stained for flow cytometry with fluorochrome-conjugated antibodies to identify platelets (CD41+/CD45-, ungated), RBCs (glycophorin A+/CD45-, ungated), granulocytes ($CD14^{mid}/SSC^{hi}$/CD45+). Right: Total number of PBMCs were determined by cell counting and values normalized to 1 mL of blood. Significance determined using two-tailed, unpaired t-test.

Conclusions:

- **Highly purified, clean cell preparations can be obtained in under 30 minutes without compromising cell recovery when using the EasySep™ RBC Depletion Reagent or EasySep™ Direct PBMC Isolation kit compared to standard ammonium chloride lysis or DGC.**

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