Automated, Medium- and High-Throughput Immunomagnetic Isolation of Extracellular Vesicles from Biofluids

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INTRODUCTION

Traditional extracellular vesicle (EV) isolation methods such as differential ultracentrifugation are time consuming, challenging to implement in clinical settings, and lack automation capabilities. To address the barriers to routine, scaled EV isolation and analysis, we developed immunomagnetic methods (EasySep™) to isolate EVs using tetraspanin surface markers. The manual EasySep™ EV isolation methods have demonstrated high specificity and recovery across biofluids. Furthermore, these methods have been shown to be highly suitable for clinical applications, particularly in the detection of blood-based EV biomarkers (McNamee et al. 2022). We have now fully automated these methods using RoboSep™-S (up to 4 samples) and RoboSep™-16 (up to 16 samples) platforms, enabling the scalable isolation of EVs for sample volumes ranging from 0.5 to 8 mL. In this study, we compared the manual isolation of EVs using the EasySep™ Human Pan-Extracellular Vesicle Positive Selection Kit (Pan-EV Kit) to the automated isolation of EVs using the same kit with either the RoboSep™-S or RoboSep™-16 instruments.

METHODS

Sample Type

Pre-processed Human Plasma: 1 mL of healthy human plasma was pre-processed to remove cells and large vesicles by centrifugation at 2,000 x g for 10 minutes followed by a second centrifugation at 10,000 x g for 30 minutes.

Western Blot

To evaluate EV recovery, the EV preparations were normalized to the same volume (e.g. EV preparations from 1 mL of plasma were resuspended in 250 µL Dulbecco's Phosphate-Buffered Saline) and 12 µL of each sample was loaded per well of protein gel. All samples were analyzed under non-reducing conditions for the detection of CD81, CD9, CD63, and albumin. Signal intensities for each marker were quantified from the same image at identical exposure times for each paired comparison. The relative EV recoveries were calculated by the signal intensity of EVs recovered by the automated methods relative to the manual method.

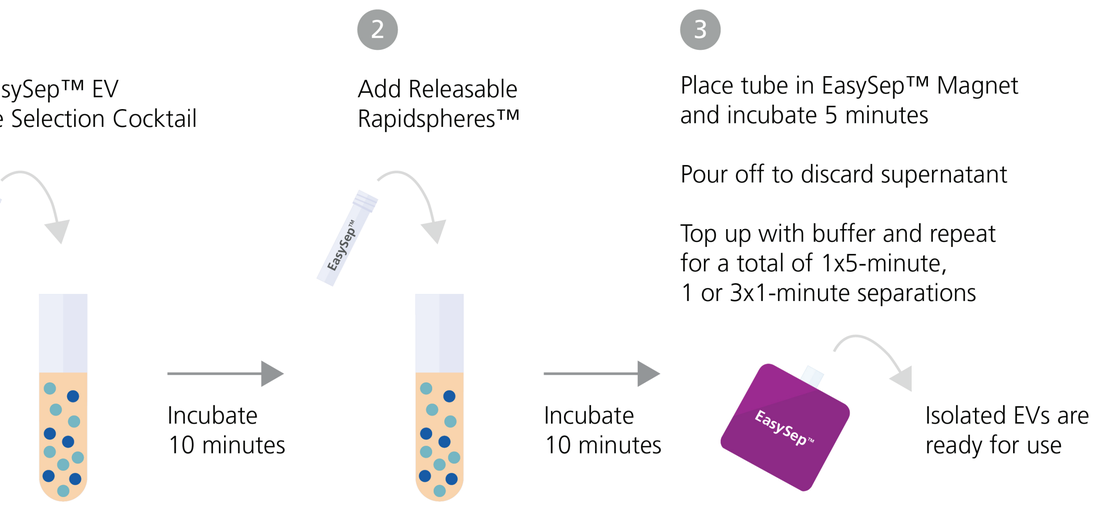

FIGURE 1. Manual EasySep™ EV Immunomagnetic Separation Protocol

EVs in pre-processed human plasma were labeled with specific antibody complexes targeting the tetraspanin markers CD63, CD9, and CD81 and magnetic particles. The desired, magnetically labeled EVs were subsequently separated from unwanted EVs using an EasySep™ magnet.

FIGURE 2. Automated EasySep™ Pan-EV Isolation Using RoboSep™-S and RoboSep™-16

(A) The RoboSep™-S instrument is designed to fully automate EV isolation from up to 4 samples at a time. **(B)** The RoboSep™-16 instrument is designed to fully automate EV isolation from up to 16 samples at a time. The automation of EasySep™ Pan-EV isolation for both instruments was performed as follows: processed human plasma, EasySep™ reagents, tips, tubes, and wash solutions were loaded onto RoboSep™-S or RoboSep™-16, guided by the on-screen prompts. A tetraspanin (CD9, CD63, and CD81)-specific antibody cocktail was added to the plasma by the instrument. Following an incubation period, magnetic particles were automatically added to the antibody-labeled EVs. The samples were subsequently transferred to the EasySep™ magnets by the instrument. After several automated washes in the magnet, the isolated EVs were recovered from the sample tube in the magnet and were ready to be resuspended for downstream applications.

RESULTS

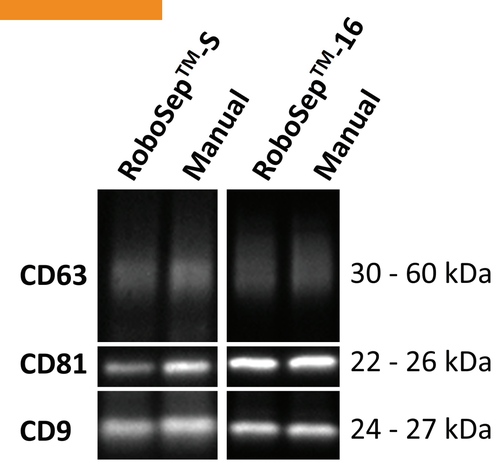

FIGURE 3. EVs Isolated Manually or with RoboSep™-S or RoboSep™-16 Expressed the Expected EV Surface Markers

EVs were isolated from pre-processed human plasma using EasySep™ Pan-EV Kit either manually or using the automated RoboSep™ -S or RoboSep™ -16 instruments. The isolated EVs were analyzed by western blot using CD9, CD63, and CD81 as detection markers.

Number of Samples	Manual EasySep™ (Minutes)	RoboSep™-S (Minutes)	RoboSep™-16 (Minutes)
1	26 - 29	37	-
4		69	56
8	-	-	63
12	-	-	83
16	-	-	101

FIGURE 4. Number of Samples and Protocol Durations for Isolating EVs Using Different Platforms

Protocol durations for EV isolation using the EasySep™ Pan-EV Kit with the manual EasySep™ magnet or automated RoboSep™-S or RoboSep™-16 platforms, covering 1 to 16 samples. The protocol durations are presented in minutes and do not include the plasma pre-processing steps.

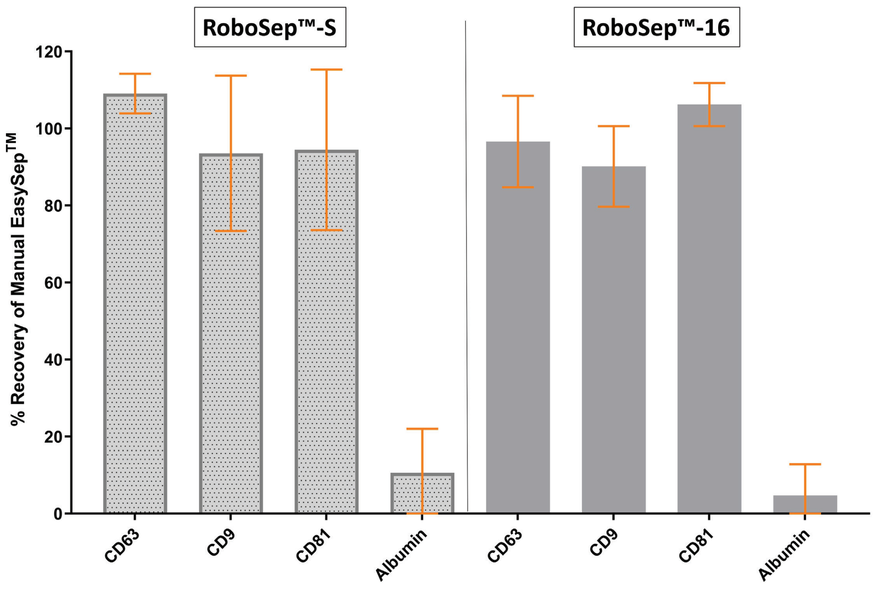

FIGURE 5. RoboSep™-S and RoboSep™-16 Automated EV Isolations Yielded Similar EV Recovery Compared to Manual EasySep™ Isolation but Lower Contaminating Albumin Levels

EVs were isolated using the EasySep™ Pan-EV Kit manually or with the automated RoboSep™-S or RoboSep™-16 instruments. The isolated EVs were normalized to an equal volume, and an identical volume from each sample was analyzed by western blot using CD9, CD63, CD81, and albumin as detection markers. The fully automated RoboSep™-S and RoboSep™-16 Pan-EV protocols recovered similar amounts of EVs compared to the manual methods, but with substantially lower serum albumin contamination (n = 3 for each platform). Error bars represent standard deviation.

Summary

- EasySep™ Human Pan-Extracellular Vesicle Positive Selection Kit provides a rapid and efficient method for the immunomagnetic isolation of EVs, compatible with both manual and automated separation platforms.
- EVs isolated with the automated RoboSep™-S and RoboSep™-16 instruments showed reduced albumin contamination compared to EVs isolated manually.
- The RoboSep™-S and RoboSep™-16 protocols outlined here can also be applied to other EasySep™ EV isolation kits, such as EasySep™ Human Extracellular Vesicle CD9, CD63, and CD81 Positive Selection Kits, EasySep™ Extracellular Vesicle PE Positive Selection Kit, and EasySep™ Human Extracellular Vesicle CD61 Depletion and Positive selection Kit.
- Automated EV isolation on RoboSep™-S and RoboSep™-16 presents practical solutions for clinical laboratories aiming to isolate EVs for the detection of disease biomarkers.

References
McNamee, N. et al. (2022) Transl Oncol 15(1):101274