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**EVOLIUCINĖ MEDICINA:
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**EVOLUTIONARY MEDICINE:
PERSPECTIVES ON HUMAN DEVELOPMENT AND HEALTH
CONSEQUENCES IN A CHANGING ENVIRONMENT**

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STANDARDISATION CHALLENGES IN EXTRACELLULAR VESICLES AND LIPOPROTEIN ISOLATION FROM HUMAN PLASMA

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Background and Aim: Cardiovascular disease (CVD) remains the leading cause of both morbidity and mortality worldwide. To enhance the understanding of atherosclerosis pathogenesis and support the development of preventive strategies, research increasingly focuses on lipoproteins and relatively understudied extracellular vesicles (EV). Extracellular vesicles and lipoproteins are abundant in human plasma and share similar physicochemical properties, which complicates their isolation and independent analysis in atherosclerosis research. This study aimed to develop clinically applicable density gradient centrifugation (DGC)-based workflows for the isolation and characterisation of EV and lipoproteins from human plasma, with a focus on reproducibility and translational applicability.

Materials and Methods: This cross-sectional study enrolled 201 participants from the national CVD prevention program. The study was approved by the Vilnius Regional Biomedical Research Ethics Committee (Approval No. 2023/9-1518-998), affiliated with Vilnius University. Plasma samples were processed using DGC with clinically feasible sample volumes. A 10–40% DGC solution was prepared, and samples were ultracentrifuged at an average of 118,700 g for 40 h at 4 °C. Fraction characterisation included total protein distribution analysis, density evaluation and Western blot assessment of EV and lipoprotein-associated markers.

Results: The developed workflow enabled the separation of EV-enriched fractions from major lipoprotein fractions. EV-associated marker CD81 was predominantly detected in fractions 5–9, whereas apolipoprotein B was localised mainly in fractions 1–4. Nevertheless, partial co-isolation with apolipoprotein A-I persisted across multiple fractions, highlighting ongoing challenges in achieving sufficient EV purity and yield from limited clinical sample volumes.

Conclusions: These findings emphasise both the potential and the current limitations of DGC-based EV isolation approaches in translational cardiovascular research. Further optimisation and standardisation of EV and lipoprotein isolation protocols are essential to improve reproducibility, facilitate cross-study comparability, and support the future integration of EV-based biomarkers into clinical cardiovascular risk assessment.

Keywords: cardiovascular disease, extracellular vesicles, lipoproteins