P008 Isolation of microvesicles and exosomes by microfiltration and estimation of normal reference range in blood plasma

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Current protocols for the isolation of microvesicles (MVs) and exosomes, which in the main focus on differential centrifugation, vary considerably. In an attempt to set a new standard, we describe a filtration protocol for isolating phosphatidylserine-positive MVs (larger than 200 nm in diameter) and exosomes. The key preparative step to successfully isolate both MVs and exosomes to a high degree of purity was a gentle sonication to break up exosome clumps. Filtration through a 100 nm pore size Millipore filter allowed for collection of exosomes in the filtrate. The larger MVs could then be recovered from the filter. Annexin V-PE MVs were sized and quantified using Polysciences Polybead Microspheres (200 nm) and BDTrucount tubes, respectively on a FACS CaliburTM flow cytometer. The normal reference range from normal human donors was found to be 0.51-2.82 $x10^5$ MVs/ml. Freeze/thawing of samples had little effect on MV counts and with age MV levels seemed only marginally reduced. Fasting status also affected MV levels, appearing up to 3-fold higher in fasting individuals. Smokers had lower MV counts and nicotine reduced MV release from THP-1 cells.