Abstract

Extracellular vesicles (exosomes) are the subject of current nephrology proteomics research as they are considered as a promising source of potential biomarkers of kidney disease. This work is focused on discovery of the most appropriate procedure for the urinary exosomes isolation. We have compared already described methods, based on different physicochemical principles of isolation: hydrostatic filtration dialysis (HFD), differential ultracentrifugation, ultrafiltration through a 100 kDa filter, or sample precipitation with Total Exosome Isolation (from urine) kit. Characterization of individual isolated exosomal fractions was performed using SDS-PAGE method (presence of contaminating proteins), western blot analysis (detection of exosomal markers TSG101, alix), nanoparticle tracking analysis (NTA, vesicle size and concentration) or transmission electron microscopy (TEM, vesicles morphology).

Due to the presence of contaminating proteins in urine samples, which could distort the results of subsequent proteomic assays, the conditions for the cleavage of undesirable proteins by proteinase K prior to their own isolation were optimized.

It has been found that the best yield and purity of the isolated exosomal fractions were provided by a process combining HFD with differential ultracentrifugation after previous incubation with an optimal amount of proteinase K.