Double Tangential Flow Filtration for purification of Urine EVs provides high particle yield and removal of THP.

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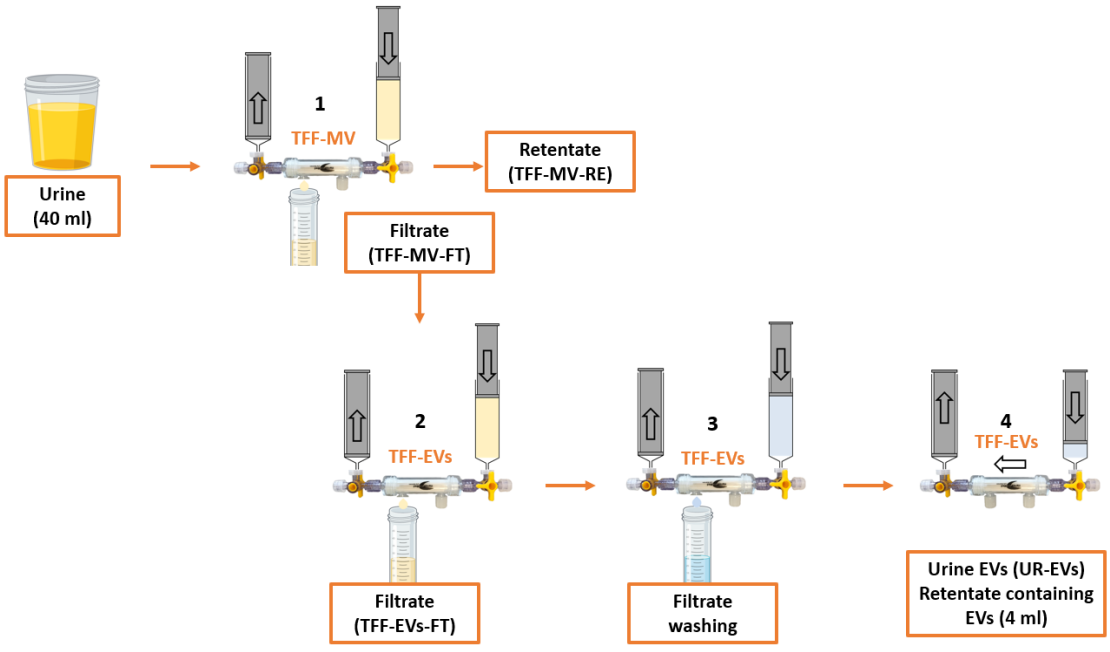
Introduction:

The study of Urine EVs and their associated biomarkers requires an efficient separation of vesicles from common contaminants. In particular, the attention is paid to the presence of the Tamm-Horsfall protein (THP), a human uromodulin that often co-isolates with the vesicles. Numerous attempts have been made to remove the THP contamination, some using chemical reagents, such as DTT or CHAPS [1;2], or trying to raise the solubility of the uromodulin [3] so as to avoid co-precipitation with vesicles. This application note shows the effectiveness and versatility of Tangential Flow Filtration in obtaining high yield of urine vesicles, free from THP contamination, in a short time and with an extremely simple, scalable and reproducible procedure.

References:
[1] Fernández-Llama, Patricia, et al. "Tamm-Horsfall protein and urinary exosome isolation." Kidney international 77.8 (2010): 736-742.
[2] Musante, Luca, et al. "A simplified method to recover urinary vesicles for clinical applications and sample banking." Scientific reports 4.1 (2014): 1-11.
[3] Puhka, M., et al. "KeepEX, a simple dilution protocol for improving extracellular vesicle yields from urine." European journal of pharmaceutical sciences 98 (2017): 30-39.

Workflow:

40 ml of human urine have been filtered through the tangential flow filter TFF-MV (200 nm pore size, HansaBioMed Life Sciences) in order to remove large particles, contaminants and debris, then the EVs contained in the flowthrough have been purified by using the tangential flow filter TFF-EVs (50 nm pore size, HansaBioMed Life sciences). EVs were finally recovered in 4 ml of PBS 1x.

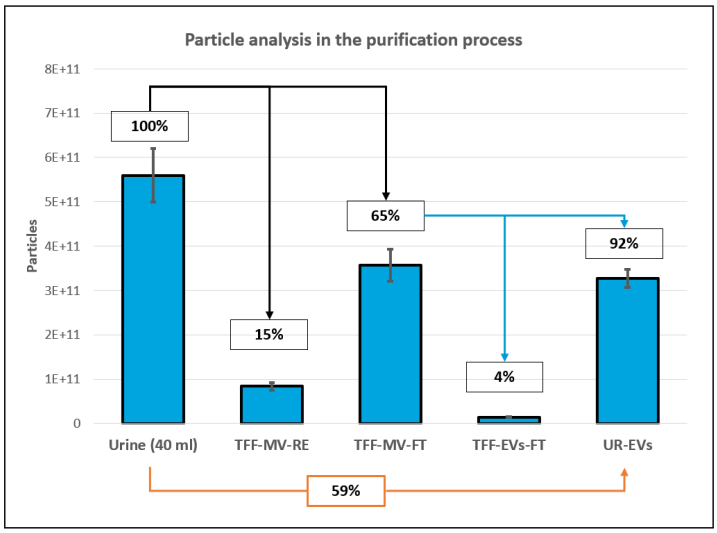


1. Filtration of 40 ml of urine through TFF-MV (200 nm pore size).
2. Purification of EVs contained in the TFF-MV filtrate by TFF-EVs.
3. Washing of the TFF-EVs retentate containing EVs with PBS 1x
4. Recovery of EVs from TFF-EVs in 4 ml of PBS 1x.

Results:

- Particle content analysis in purification process.

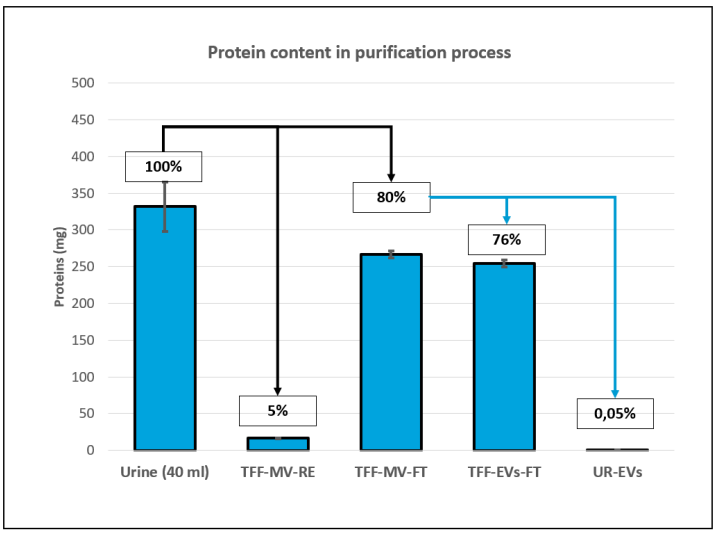
SAMPLE	CONCENTRATION (Part/ml)	St. Dev (Part/ml)	MEAN SIZE (nm)	St. Dev (nm)	VOLUME (ml)	TOTAL PARTICLES
Urine (40 ml)	1.40E+10	1.50E+09	101.2	9.5	40	5.6E+11
TFF-MV-RE	4.20E+10	4.30E+09	138.2	16.6	2	8.4E+10
TFF-MV-FT	9.40E+09	9.40E+08	95	8.2	38	3.572E+11
TFF-EVs-FT	3.80E+08	2.40E+07	84.2	8.8	38	1.444E+10
UR-EVs	8.20E+10	5.10E+09	102.9	9.4	4	3.28E+11



- Recovery of ≈ 60% of total particles.

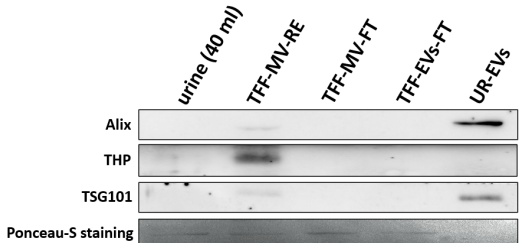
- Protein content analysis in purification process.

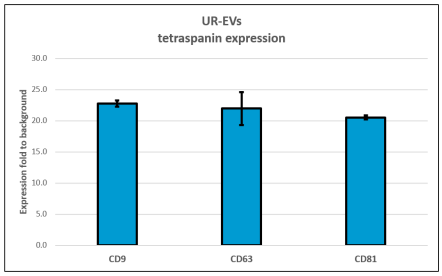
SAMPLE	PROTEIN CONCENTRATION (mg/ml)	St. Dev (mg/ml)	VOLUME (ml)	TOTAL PROTEINS (mg)
Urine (40 ml)	8.2897	0.8445	40	331.5885
TFF-MV-RE	8.4249	0.1214	2	16.84971
TFF-MV-FT	7.0241	0.1291	38	266.9166
TFF-EVs-FT	6.6863	0.1214	38	254.078
UR-EVs	0.0450	0.0325	4	0.18

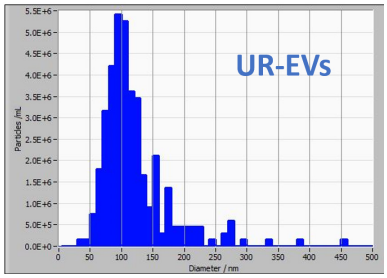


- Removal of more than 99% of contaminant proteins.

- Purifiled Urine EVs; analysis of associated markers and size distribution profiling.







- EV internal markers TSG101 and ALIX in purified EVs (UR-EVs) and THP removal.

- Detection of EV surface associated markers CD9, CD63, CD81 in purified intact EVs (UR-EVs) and size distribution profiling.

Conclusion:

The double tangential flow filtration is a relatively simple and fast protocol, which can be carried out manually on the lab bench or under the safety cabinet, providing high EV yield and purity.

- Recovery of about 60% of the total particles
- Removal of contaminant proteins, including THP.
- Turnaround time of total process: less than 10 minutes.
- No chemical treatment of the sample

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