Isolation technique	Sample Type	Sample Volume	Isolation time[I]	Recovery marker used	Recovery	Method for purity assessment	Purity assessment	Reference
Conventional techniques								
Differential Ultracentrifugation	Urine (Healthy volunteers)	25 ml	290 min	CD9, miRNAs (miR-192 and miR-1207-5p) and mRNAs (TSG101, PDCD6IP and AQP2)	±1.5 x 10^9/ml urine [CD9]	Alix and TSG101 protein bands	Strong ALIX and TSG101 protein band	Alvarez 2012 (12)
Ultracentrifugation+30% sucrose cushion		25 ml	450 min		±0.2 x 10^9/ml urine [CD9]		Strong ALIX and TSG101 protein band	
Ultracentrifugation+0.22 mm filtration		25 ml	295 min		±0.3 x 10^9/ml urine[CD9]		Strong ALIX and TSG101 protein band	
Nanomembrane ultrafiltration concentrator (Vivaspin 20;Sartorious)		15 ml	370 min		±1.3 x 10^9/ml urine[CD9]		No strong ALIX and TSG101 protein band detectable	
Standard exosome precipitation (ExoQuick-TC)		10 ml	1005 min		±0.5 x 10^9/ml urine[CD9]		No strong ALIX and TSG101 protein band detectable	
Modified exosome precipitation (ExoQuick-TC)		10 ml	1030 min		±2.7 x 10^9/ml urine[CD9]		Strong ALIX and TSG101 protein band	
Differential Ultracentrifugation	Medium from LIM1863 cells	500 uL CCM (1.5 mg protein)[I]	> 2 h	Alix, TSG101, HSP70	Immuno affinity capture > 2 fold higher than Differential Ultracentrifugation and Optiprep Centrifugation	Electron microscopy	both round-shaped 40–100 nm diameter vesicles as well as larger vesicles	Tauro 2012 (17)
Centrifugation with OptiPrep™			> 19 h				Homogenous population of vesicles, round in shape with size range of 50-100 nm	
Immunoaffinity Capture			> 5 h				EM analysis of IAC-Exos also revealed a homogeneous population of vesicles round in shape and with a size distribution in the 40–60 nm range	
Differential Ultracentrifugation	Blood plasma from healthy volunteers	5 mL	>140 min	Total protein yield	46 µg	Western Blot (for Alix, TSG101, CD9, CD63, HSP70, TFRC, and FLOT1), TEM	Western Blot: CD63, CD9, HSP70, FLOT1 and TFRC were detected; TEM: contain sparsely scattered exosomes	Kalra, 2013
Differential Ultracentrifugation and Centrifugation with Sucrose and OptiPrep™		25 mL	>22 h		-		Western Blot: CD63, anti-CD9, HSP70, FLOT1 and TFRC were detected; TEM: particles in the range of 40–100 nm	
EpCAM-based immunoaffinity pull-down		5 mL	>5 h		225 µg		Western Blot: CD9 and CD63were detected; TEM: small membranous vesicles in the range of 40–70 nm	
Differential Ultracentrifugation	Blood serum from healthy volunteers	1 mL	>4 h	Measurement of a panel (375) of miRNAs.	93 miRNAs above detection limit (Ct<35)	Difference in miRNA expression between isolation methods	miR-92a andmiR-486-5p showed statistically different levels between the two methods (fold change 2.9 and 4.2, respectively; p-value < 0.00048). Clustering of 17 miRNAs revealed different expression pattern between isolation methods.	Rekker, 2014
ExoQuick Precipitation (EQ)			Overnight incubation + 30 min centrifugation		128 miRNAs above detection limit (Ct<35) verage miRNA detection threshold levels were 1.7 cycles lower in EQ samples			

On-chip isolation								
On-chip immunological Capture	Serum sample	400 µL	Around 30 min	Percentage of total RNA recovered from chip and effluent, using CD63 and IgG as a control	42-94%	RNA analysed through Bioanalyser. No control for proteins. EM imaging	No ribosomal RNA found (no cellular contamination) Only 3% of the projected area diameters of microvesicles from serum were greater than 100 nm	Chen 2010 (21)
On-chip immunological Capture			60min	Western Blot (CD63,Rab 5) RNA extraction and openarray miRNAs analysis	15-18µg total proteins 10-15 ng nucleic acids	EM imaging	EM images show the presence of both small sized vesicles of ~30 nm diameter and larger vesicles of up to 300 nm	Kanwar 2014 (20)
On-chip immunological Capture			100 min	Retention rate on magnetic beads, iGF-1R quantitative detection	>99.9% beads recovery iGF-1R detection sensitivity 100 times higher than standard ELISA	TEM imaging of beads functionalized with EpCam,α-IGF-1R, CA125. Nanosight measurements	TEM images show densely coated vesicles on antibody beads in patient sample, and almost no vesicles on the negative control beads without specific antibodies were observed. Nanosight measurements show narrower peak size around 50-100nm for on-chip exosomes compared to ultracentrifugation.	He 2014 (22)
On-chip immunological Capture with plasmonic sensor	Clinical ascite samples (after 0.2 µm filtration)	150 µL	Around 60min depending on incubation period	mRNA content of trapped exosomes by qRT-PCR (Epcam, CD24) (Ca0V3 cells)	Epcam: 2-3% mRNA levels % of GAPDH CD24: 3% mRNA levels % of GAPDH	TEM imaging	TEM images of nplex with cup-shaped particles in the correct size range. Negligible binding when a control antibody was used.	Im 2014 (23)
On-chip filtration	Blood	240 µL	Around 100min	Relative recovery measured in terms of protein content from ultracentrifuged samples, then pass through electrofiltration	1.5% recovery of EV	TEM images Western blot	TEM images of pressure filtered and electro-filtered samples show presence of cup-shaped vesicles. CD9 signal compared to other coexisting proteins, indicating an increase in EV-specific proteins	Davies 2012 (25)
On-chip nanosieving	Mixture of BSA, 83nm liposomes, 500nm beads	10-100 µL	10min for isolation on-chip, 24h for recovery	Retention rate on 83nm liposomes (based on fluorescent intensities of the injected and collected samples)	60% retention rate on 83nm liposomes for 10-30 µl of sample volume	Retention rate on 83nm liposomes and 500nm beads	60% retention rate on 83nm for 30 µl of sample volume, and 10% 500 nm bead retention	Wang 2013 (7)
Inertial focusing / Buffer exchange (RinSe)	Lysed blood spiked with	~1mL	Around 10 minutes on-	Bead count	~100%	Microscopic observation,	Large microvesicles (1 µm) did not enter the sample collection channel.	Dudani 2014

	cancer cell culture supernatant		chip (4h incubation with beads)	RNA measurements (Bioanalyser) using control beads or anti-CD63 beads	2.6 fold more RNA collected with antiCD63 vs control beads. 9 fold higher peak in small RNA size fragments for anti-CD63.	staining of large microvesicle, Size measurements on Zetasizer (Dynamic Light Scattering)	Collected samples within expected size range (75.4nm +15.5nm) Inlet solution peak at (3.1 nm + 0.8 nm)	(27)

Table S1. Comparison of isolation techniques. CCM stands for concentrated culture medium. [I] Isolation time from start of protocol until purified concentrate of exosomes is obtained. [II] CCM was concentrated till 1.11 g/mL proteins by centrifugal filtration using a 5 K NMWL membrane filter.