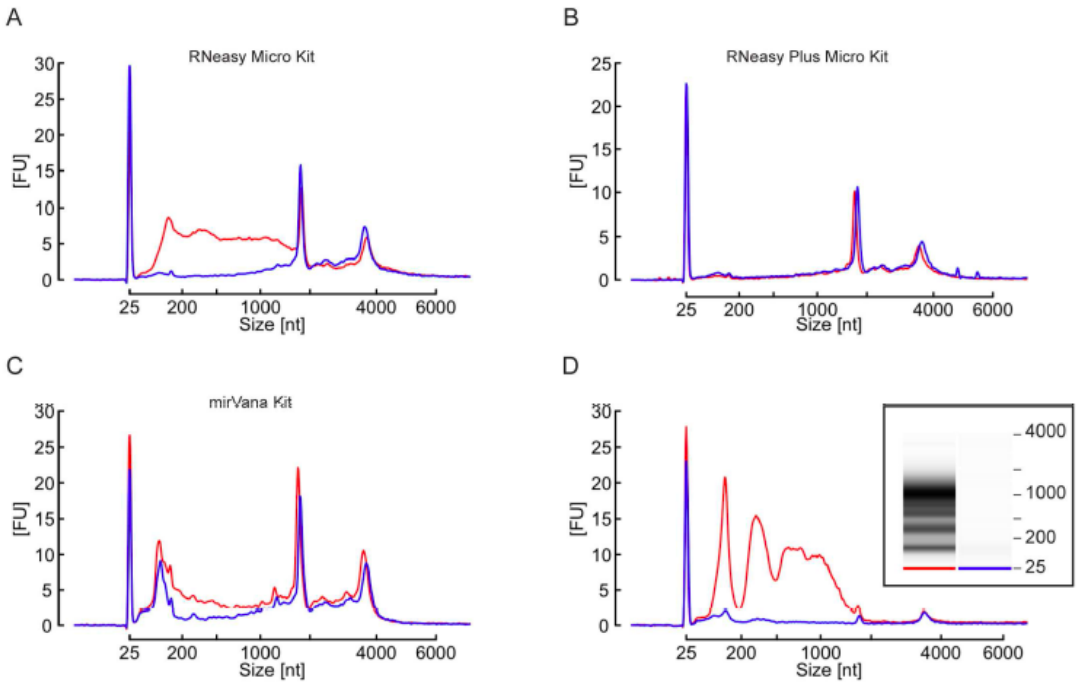
Miranda et al Kidney Int 78: 191-199, 2010

Supplementary data and figures:

Various methods for the removal of extraneous DNA (i.e. DNA on the outside of exosomes) from samples were also investigated. The use of DNase digestion of isolated microvesicle pellets followed by extraction of microvesicle nucleic acids using the RNeasy Micro kit was found to efficiently remove contaminating gDNA (Supp Fig. 1A). We also examined the use of the RNeasy Plus Micro kit, which is designed to remove gDNA from samples (see methods). This kit efficiently removed contaminating extraneous DNA similar to when DNase digestion of the microvesicle pellet was carried out pre-lysis (Supp Fig. 1B). The use of the acid phenol/chloroform extraction method (such as the *mir*Vana RNA extraction kit) is thought to remove genomic DNA via phase separation whereby DNA and proteins are separated into the interphase and organic phases respectively (1). Our results demonstrated most extraneous DNA was removed from the sample but that the use of DNase treatment prior to microvesicle lysis in addition to the *mir*Vana kit consistently resulted in a further decrease in DNase digestible material (Supp Fig. 1C). Analysis of various urine and serum samples revealed that varying degrees of extraneous DNA might accompany microvesicle isolation. In serum we consistently observed nucleic acid profiles that appeared similar to 'apoptotic body' like ladders (Supp Fig. 1D and pseudo gel inset from a serum-derived microvesicle isolation) and the presence of small but detectable 18S and 28S rRNA peaks. Although it has previously been suggested that such apoptotic bodies are confined to vesicles (2) we demonstrate here that this nucleic acid material which co-isolates with microvesicles is susceptible to DNase digestion when the microvesicle pellet is treated with DNases prior to microvesicle lysis. This suggests that this DNase susceptible 'apoptotic body' like ladder material is either i) not confined within vesicles or ii) is confined to delicate vesicles that are easily disrupted (unlike the majority of microvesicles which appear stable).

Methods:

Isolation and DNase digestion using the RNeasy Micro kit was performed as described in the main methods section. For the *mir*Vana miRNA isolation kit (Ambion, CA), RNA was isolated according the manufacturer's instructions and eluted from the column using 100 µl elution buffer. The eluted RNA then underwent processing by the RNeasy MinElute Cleanup Kit (Qiagen, CA) and eluted in a final volume of 16 µl. For serum microvesicle isolation, serum was separated from whole blood using serum separator tubes (Becton Dickinson and Company, CA). The serum then underwent centrifugation at 17,000*g* at 4°C for 20 min. The supernatant was processed through a 0.8 µm filter and the filtrate ultracentrifuged at 118,000*g* for 70 min at 4°C. The pellet was washed in PBS and re-centrifuged at 118,000*g* for 70 min at 4°C. The pellet then underwent DNase digestion as described below.



Supp Figure 1: Analysis of various methods for the removal of extraneous DNA contamination. **A)** DNA on the outside of urinary microvesicles can be removed via DNase digestion of the microvesicle pellet prior to lysis followed by nucleic acid extraction using the RNeasy Micro kit (Qiagen,CA). Red – without DNase digestion prior to microvesicle lysis, blue - with DNase digestion prior to microvesicle lysis. **B)** Using the Qiagen RNeasy Plus Micro kit which is designed to remove gDNA during the RNA isolation procedure we show that this kit was as efficient at removing extraneous DNA from the sample as DNase digestion on the outside of microvesicles prior to lysis and nucleic acid extraction. red – extraneous DNA removal via the RNeasy Plus Micro kit, blue – extraneous DNA removal via the RNeasy Plus Micro kit in addition to extraneous DNase digestion. **C)** Using the Ambion *mir*Vana kit acid phenol/chloroform method the profiles for RNA extraction showed similar peak trends but with reduced peak height when DNase digestion was carried out on the outside of microvesicles pre-lysis in addition to the use of the *mir*Vana kit. Red – *mir*Vana kit alone, blue – *mir*Vana kit with extraneous DNase digestion. Also note the characteristic large peak on the LHS of the trace representing small/miRNA which the *mir*Vana kit is designed to co-purify. **D)** DNase susceptible 'Apoptotic body' like ladders which may co-isolate with serum-derived microvesicles (see red profile and inset of 'pseudo' gel) can be removed from the sample via DNase digestion on the outside of microvesicle prior to microvesicle lysis (Qiagen RNeasy Micro kit was used for extraction). Ribsosomal RNA peaks are also evident in serum-derived microvesicles. The same sample was used in the generation of Supp Fig. 1A to 1C to allow for comparison of the different isolation techniques.

References:

1. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-9, 1987

2. Saraste A, Pulkki K: Morphologic and biochemical hallmarks of apoptosis. Cardiovasc Res 45:528-37, 2000