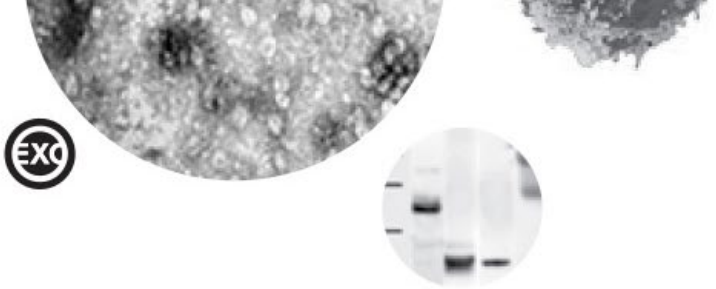
FROM TRASH BIN TO GOLD MINE.

THE TRUE STORY OF EXOSOMES.

SBI System Biosciences
Harnessing innovation to drive discoveries



1 Introduction To Exosomes

2 EVs as Biomarkers of Disease States

3 Harnessing EVs as Biomarkers

INTRODUCTION

Pop open any college biology textbook from the early 1990s. Move over to the textbook's index and search for "exosomes". Flip to the right page.

You're sure to see that somewhere on that page is an explanation that exosomes are, in effect, cellular trash receptacles.

Derided for years as nothing more than backwash no one wanted to touch, exosomes are now at the bleeding edge of radical new diagnostic and therapeutic research.

They're the new "it".

This is their amazing story. About what they are, what they can do for us, and how you can harness their power for research, diagnostic, and therapeutic purposes that will hopefully change the world of science.

Chapter 1
Introduction to Exosomes

What Are Exosomes?

Exosomes are extracellular vesicles of endosomal origin that are between 30 to 100 nanometers in size.

One key factor distinguishes exosome formation from the way by which microvesicles come into existence. The main difference is that while exosome formation is dependent upon the endosomal system, microvesicles (100-1000 nm in size) originate from the plasma membrane (Diagram 1.1).

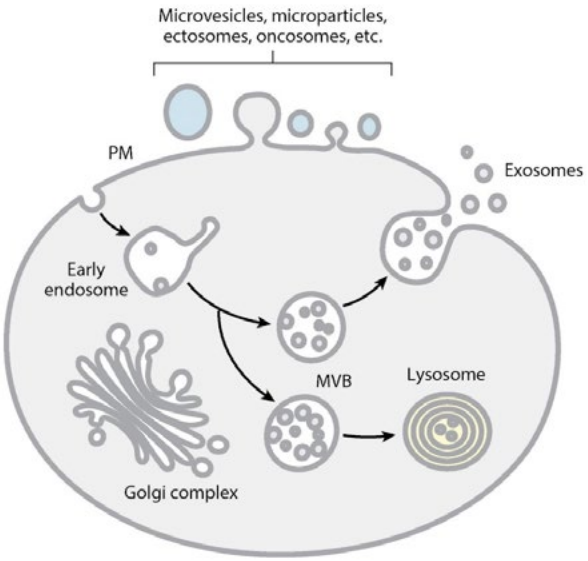


Diagram 1.1: Exosome Formation.
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Exosome Biogenesis

At its very core, the exosome biogenesis pathway is relatively straightforward.

Exosome biogenesis begins after the invagination of the cell membrane. The inward budding of the cell membrane results in the formation of early endosomes.

In this pathway, the early endosomes eventually mature into late endosomes/multivesicular bodies (MVBs).

Within the MVBs, the exosomes originate as intraluminal vesicles (ILVs) (Image 1.1).

Exosomal biogenesis concludes when the MVB is transported to, and fuses with, the plasma membrane; whereupon the ILVs are released into the extracellular milieu.

Upon their release, the ILVs are then called exosomes.

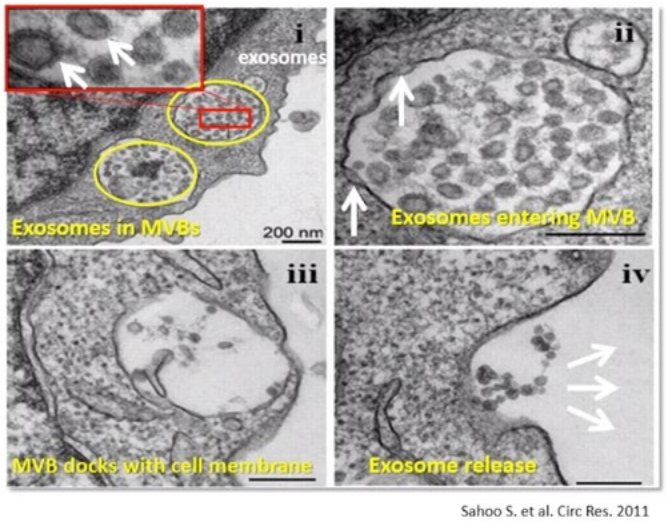

Image 1.1: Exosome Biogenesis

Exosome Uptake

While the exosome biogenesis pathway is relatively linear at its crux, exosome uptake is a completely different story.

That's because, after their release into the extracellular environment, the pathways for exosomal uptake are multifaceted.

First and foremost, exosome uptake can either be a local or distant process. In other words, while neighboring cells are able to interact with or utilize the newly released exosomes, the exosomes may be transported to more distant sites via the bloodstream as well.

In either case, the mechanisms for exosome uptake thereafter are even more diverse. They include everything from vesicle-cell fusion to endocytosis to important ligand-receptor interactions (Diagram 1.2).

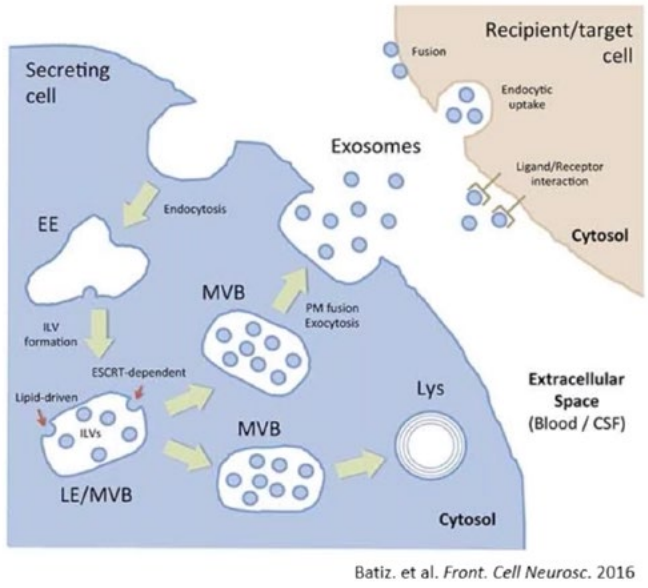

Diagram 1.2: Exosome Uptake & Release

Exosome Size and Features

As mentioned before, an exosome's size ranges from 30-100 nanometers and these vesicles can be secreted by a wide range of cells; where their composition will depend strongly on their cell of origin.

Of note, virtually all mammalian cells that have been studied exhibit exosome-based communication.

And as with these cells, an exosome's contents are likewise going to be quite varied. This is because exosomal contents can include proteins such as enzymes, as well as mRNAs and ncRNAs, lipids, and much more (Diagram 1.3)

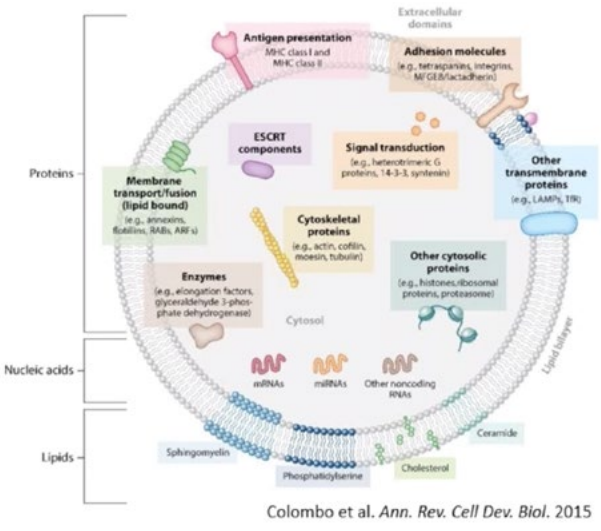

Diagram 1.3: Exosome Features.

EVs as Biomarkers in Cancer

It's this connection between the cell of origin and an exosome's contents that's so key to the burgeoning field of exosome research; including the interplay between exosomes and cancer.

Scientists are starting to understand that exosomal contents can serve as useful cancer biomarkers or even biomarkers in plenty of other ailments, such as neurodegenerative diseases.

As you might expect, these and other discoveries have led to a rapid rise in exosome-based cancer biomarker research in recent years and a lot of promise for the detection, monitoring, and treatment of all sorts of neoplastic conditions (Diagram 1.4).

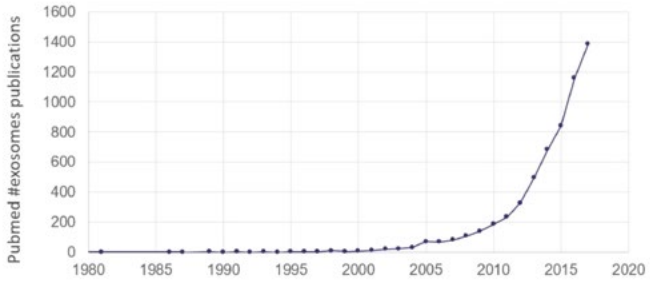

Diagram 1.4: Growth of Exosome Research.
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Why EVs as Biomarkers?

So why are extracellular vesicles, including exosomes, such useful biomarkers then?

The major reason for this is quite simple. It's because they're present in virtually all the biofluids you can think of (Diagram 1.5). Extracellular vesicles can be found in fluids such as:

- Serum, plasma
- Urine
- Seminal fluid
- CSF
- Saliva
- Tears
- Breast milk

This ubiquity makes extracellular vesicle-based diagnostics that much more practical in terms of ease

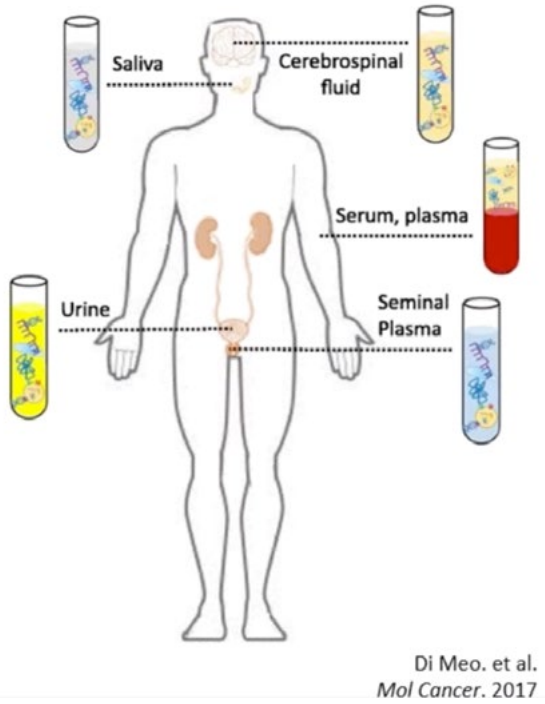

Diagram 1.5: Human Body Fluids Where EVs Are Found.

of use, speed, and minimally invasive procedures.

Moreover, extracellular vesicles can be used as biomarkers for more than just initial diagnostic purposes. These vesicles, including exosomes, can act as overall prognostic indicators as well as predictors of response to treatment, such as cisplatin.

Consequently, the one-size-fits-all approach to treatment will hopefully be part of a bygone era soon enough.

Consequently, the one-size-fits-all approach to treatment will hopefully be part of a bygone era soon enough.

EVs' Potential Goes Beyond Diagnostics

In other words, we're on our way to customizable, patient-centered, treatment approaches.

For example, we may be able to identify the proteins or microRNA we need to alter in a given patient. Then that same patient's exosomes can be isolated, modified with the appropriate siRNA or proteins, and then placed back into the patient for treatment (Diagram 1.6).

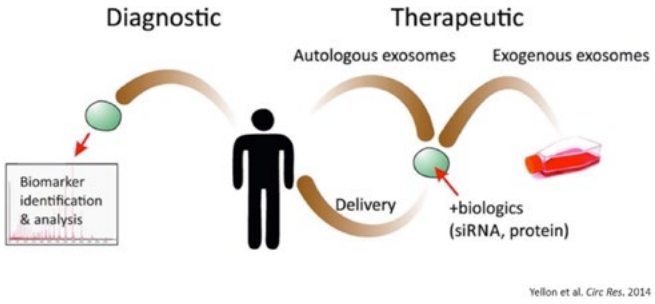

Diagram 1.6: Diagnostic and Therapeutic Uses of Exosomes

Chapter 2
EVs as Biomarkers of Disease States

Exosomal Protein Biomarkers for Spinal Muscular Atrophy

Given the entire discussion above, it's evident that extracellular vesicles can play an important role in health and disease.

Let's take a more specific look at how this is the case with a review of some recent and interesting exosome research.

We'll start with a study published in Scientific Reports, which showcases the importance of exosome-based protein biomarkers.

In this study, researchers took a look at the connection between spinal muscular atrophy (SMA), survival motor neuron (SMN) genes, exosomes, and survival motor neuron (SMN) protein.

SMA is a genetic disease. It's caused by the homozygous mutation or deletion of the SMN1 gene; where the severity of the disease is then modulated by the number of copies of the highly similar SMN2 gene (Diagram 2.1).

The end result in SMA is that there's a significant deficiency of full length SMN protein; and the severity of SMA is inversely correlated with the amount of SMN protein being produced.

As you may be aware, SMA is a highly debilitating neuromuscular disorder. And although antisense oligonucleotide therapy (Spinraza) was recently approved for it, there's still a lot of room left for an

The end result in SMA is that there's a significant deficiency of full length SMN protein.

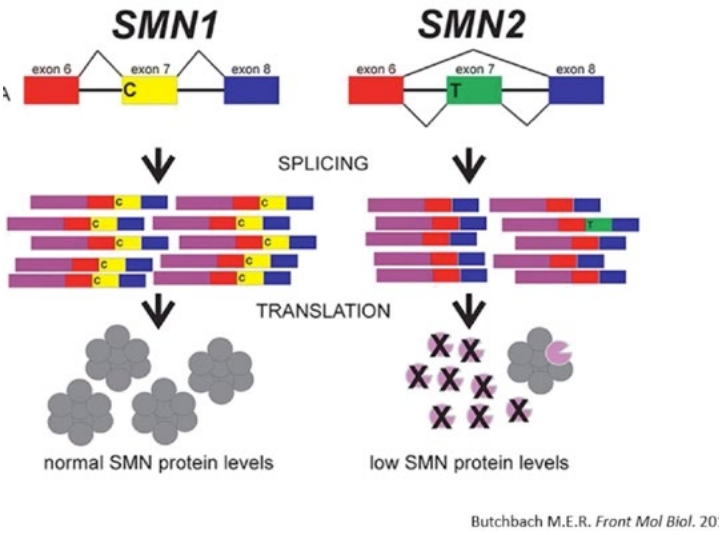

Diagram 2.1: Survival Motor Neuron Protein in SMA.
At least with respect to genetic disorders, the most common cause of death in newborns is spinal muscular atrophy (SMA). SMA is characterized by the deficiency of full-length survival motor neuron (SMN) protein, the degeneration of α-motor neurons, and subsequent skeletal muscle atrophy. SMA is caused by homozygous deletion or mutation of the SMN1 gene. While people do have a highly similar copy of the SMN1 gene (SMN2), a synonymous point mutation therein results in the production of an mRNA devoid of exon 7. SMNΔ7 protein is thus unstable and is unable to function like the full-length SMN protein.

objective and minimally-invasive way by which disease severity, prognosis, and even therapeutic direction can be more accurately ascertained.

This is where the measurement of exosome-derived SMN protein biomarkers comes in via this study.

The examination of protein found in TCA precipitates from numerous cell lines revealed that SMN protein is normally released from cells and into the extracellular environment in cell culture media (Diagram 2.2).

Given that, it was then important to determine if SMN protein could be detected in extracellular vesicles, especially exosomes. As a result, the authors performed electron microscopy using immunogold labeling. With this, they determined that the exosomes clearly contained SMN protein while there was very little SMN protein outside of any type of vesicle.

In brief, this means that cells naturally release SMN protein via exosomes (Diagram 2.3).

Then, using a mouse model of SMA, the scientists determined that the quantity of SMN protein within exosomes clearly reflected the intracellular contents and disease state. Healthy mice contained high levels of SMN protein in their exosomes while affected mice had low or undetectable levels of SMN protein.

Similar results were found in healthy controls, carriers, and SMA patients. Meaning, carriers had lower levels of exosome-based SMN protein than control while those with SMA had barely detectable levels of SMN protein within exosomes (Diagram 2.4).

Further still, the authors quantified the levels of exosomes themselves. Importantly, they

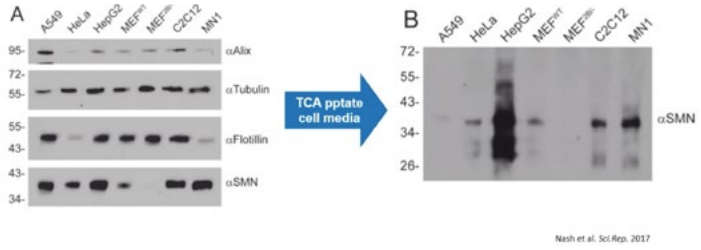

Diagram 2.2: SMN Protein is Released into the Extracellular Milieu. *Cells release SMN protein into the extracellular environment. According to the study's authors: "Various cell lines were plated in 35 mm dishes for 24 h. Media was removed, the cells lysed with protein loading buffer to analyse intracellular protein content (Panel A), and the media subjected to TCA precipitation to analyse extracellular protein content (Panel B). Equal volumes of protein sample were subjected to SDS-PAGE, transferred to a nylon membrane and probed by immunoblot with antibodies to Alix, tubulin, flotillin, or SMN. Data is representative of n = 3.*

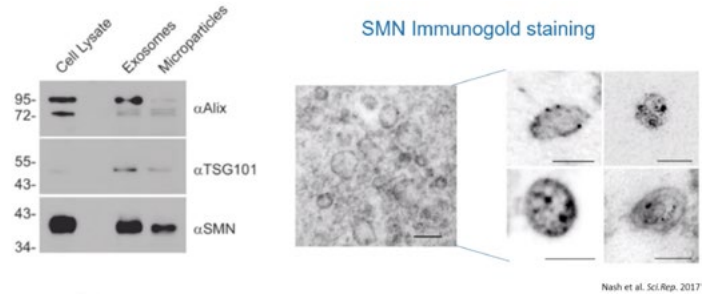

Diagram 2.3: SMN Protein Released into the Extracellular Milieu is Confined in EVs. *The authors started out by isolating microparticles and EVs from "A549 cells, grown in medium supplemented with vesicle-depleted FBS, using differential centrifugation and their size profiles were determined using nanoparticle tracking analysis. Protein from extracellular vesicles (5 µg) was subjected to SDS-PAGE and the resulting immunoblot was probed for Alix, TSG101, and SMN… Immunogold labeling of SMN protein was used to demonstrate the presence of SMN protein within A549-derived exosomes (scale bar represents 100 nm)…and higher magnification of electron microscopy images showing SMN protein contained in exosomes [was performed] (scale bar represents 100 nm)."*

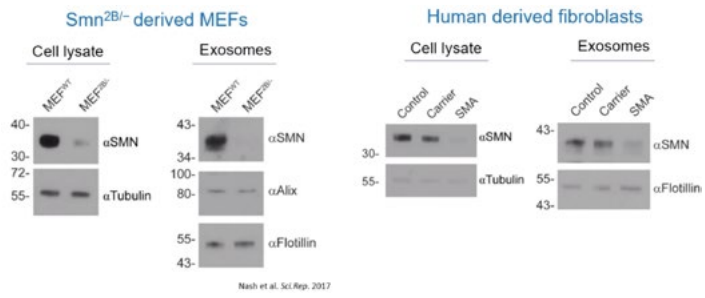

Diagram 2.4: Quantity of SMN Protein within Exosomes Correlates with Host Cell Protein Expression Levels.

observed an increased number of exosomes in the serum of patients with SMA as compared to control.

Overall, patients with SMA can be expected to have elevated levels of exosomes in their serum but lower quantities of SMN protein within those same vesicles (Diagram 2.5).

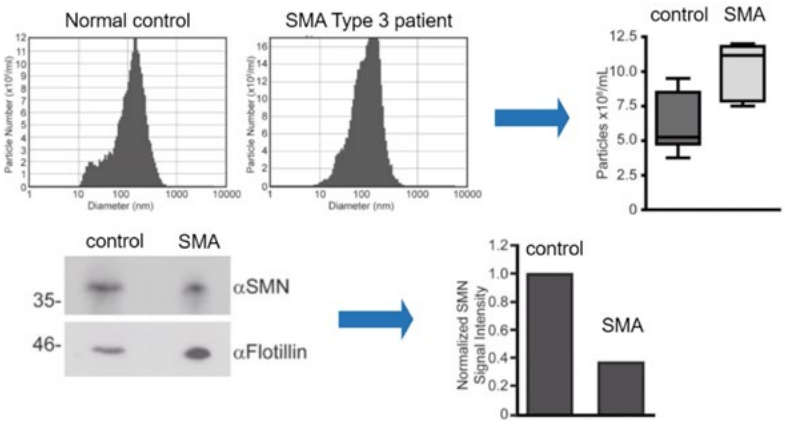

Diagram 2.5: SMN Protein Levels in Human Serum Derived EVs Reflect the Disease State.

These results have important implications. They suggest that there may be a practical way by which we can diagnose and monitor SMA, including response to treatment, via the quantification of exosome-based SMN protein biomarkers and the concentration of serum-derived exosomes themselves. All of this, using minimally invasive techniques—no less.

Exosomal miRNA Biomarkers for Multiple Sclerosis

That's not the only way by which a biomarker, contained in exosomes, can be used to assess disease states.

Other than proteins, another major component we can find in exosomes is microRNA (miRNA). A recent paper assessed exosomal miRNA biomarkers and signatures in multiple sclerosis in order to determine if they could accurately reflect a patient's disease status.

As you might be aware, multiple sclerosis (MS) is a chronic inflammatory, demyelinating, neurodegenerative disorder.

The clinical phenotypes of MS are numerous and include the following:

- Relapsing-remitting MS (RRMS)
- Primary progressive MS (PPMS)
- Secondary progressive MS (SPMS).

While RRMS is the most prevalent form of MS, comprising over 70% of all cases, within 10-15 years of disease onset the majority of patients with RRMS will progress to SPMS. Unfortunately, SPMS is a phase of MS that is known for its gradual worsening of clinical signs and symptoms and a heterogeneous or complete lack of response to existing therapy.

As of now, there's no single definitive test for MS. Therefore, it can be difficult to diagnose this disorder as its diagnosis and the monitoring of disease progression relies on multiple (sometimes subjective) clinical parameters; such as physical exams, MRI, and assessments of CSF.

Ongoing research indicates that circulating exosomes are promising candidate biomarkers for MS since they may contain everything from nucleic acids to proteins and can cross the blood-brain barrier. Biomarkers that can be used to identify patients who are able to benefit from therapy the most.

In other words, there's a big need for objective, easy, even minimally invasive liquid biopsies and markers (like miRNA biomarkers) that can stratify patients with MS.

Consequently, the authors of one study hypothesized that serum exosomal miRNAs could represent a useful blood-based assay to detect and monitor MS.

The researchers started out by isolating and characterizing extracellular vesicles (EVs) derived from the serum of controls and patients with MS.

Using 1 mL of serum from each individual, the authors treated the serum samples with RNaseA in order to remove any unprotected circulating RNA, isolated the exosomes using size exclusion chromatography, and analyzed them via nanoparticle tracking analysis and transmission electron microscopy.

The presence of exosomal markers like CD81, CD63, and Alix was confirmed (Diagram 2.6).

RNA extraction yielded an RNA profile we'd expect for exosomes; where the bioanalyzer showed a typical peak between 25-200 nt without any indication of ribosomal RNA. The authors also compared miRNA profiles between samples with and without RNAse pre-treatment (Diagram 2.7).

The researchers were able to identify a set of exosomal miRNAs that were differentially expressed between subtypes of MS.

Furthermore, they assessed receiver operator characteristic (ROC) curves and AUC measures for each candidate miRNA; for both RRMS and S/PPMS as compared to control. By combining different sets of miRNAs, the researchers created miRNA signatures that improved the ability to distinguish between subtypes of MS and control.

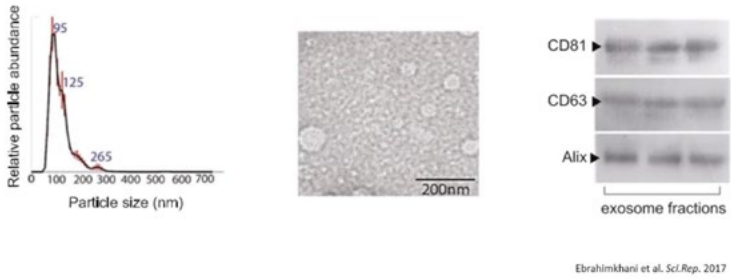

Diagram 2.6: Identification & Characterization of Serum-Derived EVs.

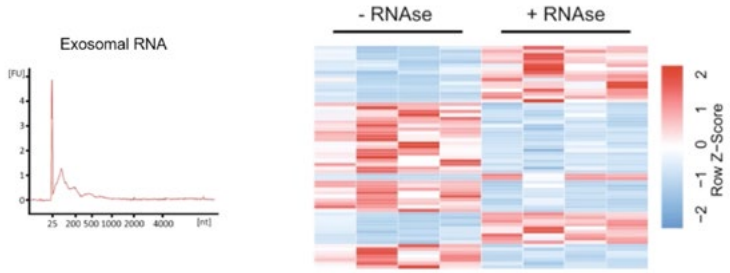

Diagram 2.7: Serum Exosomes Carry Unique miRNA Signatures.

And while numerous candidate miRNAs have been proposed as potential biomarkers for MS in the past, the biomarkers identified in this study were, by and large, completely novel. This might be because of various differences between the unique constituent profiles of exosomes as compared to free circulating miRNAs.

Most importantly, this research showed that serum exosomes are a novel source of potentially useful miRNA biomarkers.

Chapter 3
Harnessing EVs as Biomarkers

Best Practices

Perhaps all of this interesting research has got you excited about extracellular vesicle isolation and the potentials thereof.

There are a few things you may want to consider though.

The first of which revolves around the confusion about, and lack of consensus of, how to distinguish different subsets of EVs from one another.

Luckily in 2014, the International Society for Extracellular Vesicles (ISEV) came up with the minimal experimental requirements for the definition of extracellular vesicles.

In summary, the guidelines state that—if you're going to characterize single vesicles—you should use two different yet complementary techniques when doing so. For instance: electron or atomic force microscopy to have an image of the EVs in order to assess their size and shape; and single particle tracking (NTA) where one can assess both the size and count.

ISEV's requirements for general characterization include:

- ≥ 3 positive protein markers for EVs, with at least one being a:
  - Transmembrane/lipid-bound protein
  - Cytosolic protein
- ≥ 1 negative protein marker such as argonaute or histones that shouldn't be contained within exosomes.

Given that, let's presume you'd like to focus your extracellular vesicle isolation efforts specifically on exosomes. Maybe you're on the hunt for exosomal biomarkers or you're looking to actually use known exosome-based biomarkers for a downstream purpose.

What are some of the other important things you should keep in mind when harnessing these exosomes as biomarkers?

Well, for starters let's consider a basic exosome research workflow (Diagram 3.1).

Diagram 3.1: Exosome Research Workflow.
From SBI

Challenges of EV Biomarker Discovery

As you can imagine given the aforementioned discussion, there are numerous challenges with respect to the use of EVs for biomarker discovery.

The two main components of EVs are RNAs and proteins; we'll start with the challenges surrounding the former and then move on to those involving the latter.

Exosomal RNA is mainly comprised of species between 20-200 nt; including microRNAs, lncRNAs, mRNAs, and so on (Diagram 3.2).

One of the challenges here is that concentrations are going to be very low. At the same time, we need to be sure that we're identifying the RNA found within exosomes instead of other possibilities, like freely circulating RNA.

Additionally, it's important to take into account that the make-up of exosomal RNAs is going to vary a lot between individuals and between sample types.

As a case in point: in one paper, exosomal RNA was assessed from serum and urine samples. There was a clear distinction between the two sample types. For instance, the serum sample was far richer in microRNAs than the urine sample. Yet there was plenty of intraindividual variabilities as well (Diagram 3.3).

At SBI we generated some interesting data using our Exo-NGS service with respect to all of this. We found that, just as before, the composition of exosomal RNA can vary enormously between different types of samples (Diagram 3.4).

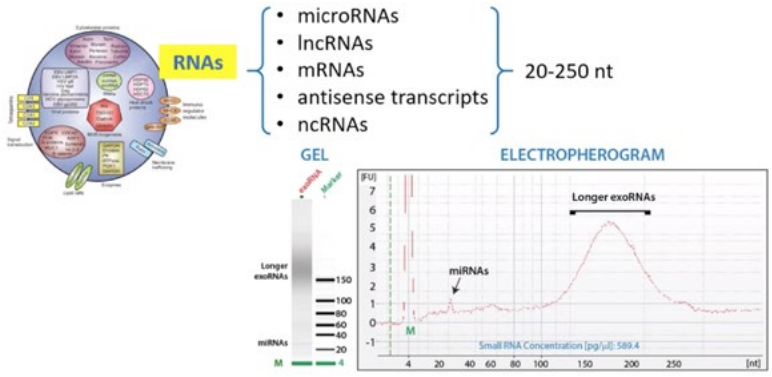

Diagram 3.2: Exosomal RNA Challenges: Concentration & Composition.
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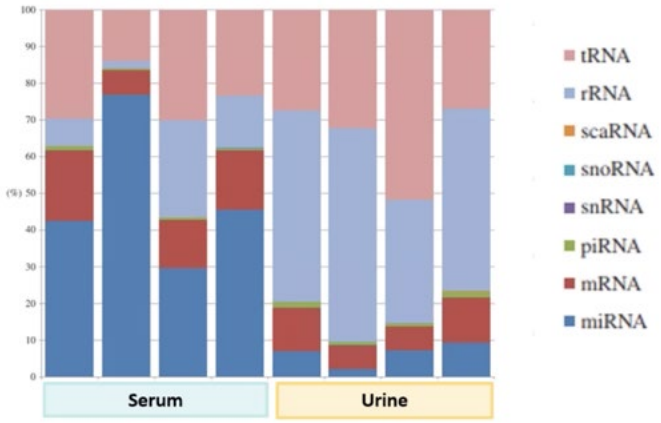

Diagram 3.3: Class Make-up of Exosomal RNAs.

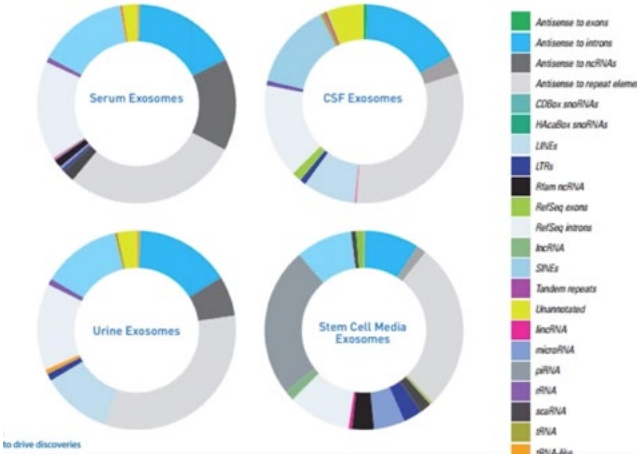

Diagram 3.4: Class Make-up of Exosomal RNAs in Body Fluids.
From SBI

Thus, the number of samples that one uses to try to assess or come up with a novel biomarker is very important.

Just as well, the way one treats the sample, the processing, and the isolation; all of these factors are going to affect the final make-up and composition of the exosomal RNA and thus the entire process of biomarker discovery.

As for proteins, there's a wide range of them within exosomes as well. They include cytoskeletal proteins, enzymes, and signal transduction proteins among many others (Diagram 3.5).

The current approaches used to characterize the proteins in EVs include western blotting, protein arrays, and mass spectrometry.

Western blotting is easy to perform and there are plenty of antibodies available. So if you know which protein you're targeting, western blotting is great. However, if you're involved in biomarker discovery then you should keep in mind that the use of western blotting is pretty limiting; it's low-throughput and you can only assess the IDs of known markers.

Protein arrays, however, have the advantage of being scalable; with low to mid-throughput and there are many commercially available panels. That being said, protein arrays are expensive, there's quantitation bias and, again, you're only able to ID known markers.

Finally, there's mass spectrometry; which is the most comprehensive approach. It's a method of unbiased protein quantification. Of course, it's quite complex, requires expertise, and is of a high cost. Not to mention: it's extremely sensitive to contaminating proteins in the sample.

Place all of this in the context of the typical protein profile in serum and plasma; which contains more than 50% albumin. In contrast, the pool of low abundance proteins makes up roughly 5%-10% of the total proteins (Diagram 3.6). So it's very difficult to find these biomarkers within samples such as these, given their propensity for large quantities of background and contaminating proteins.

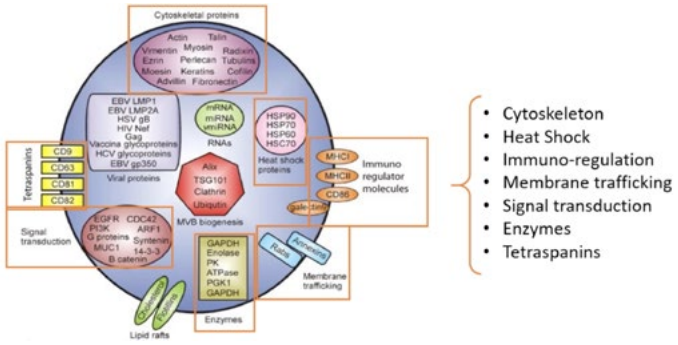

Diagram 3.5: Exosomal Proteins.
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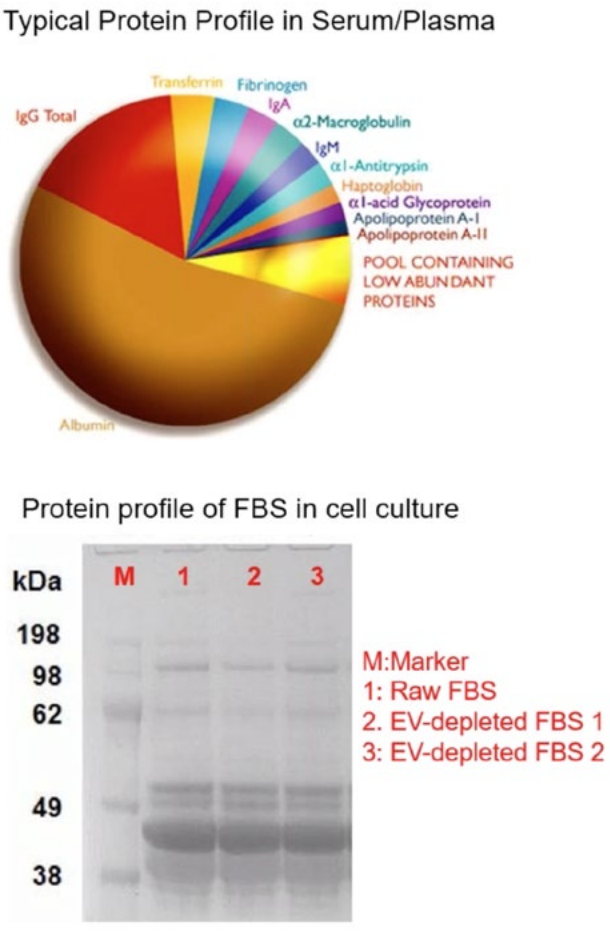


Diagram 3.6: What's in Your Sample? Challenges of EV Protein Biomarker Discovery.
Taken from powerpoint slide show. Please provide a citation.

To get a more practical read into the implications of this for your research, we generated some data about this in-house, where we compared the mass spec of either a standard prep or an ExoMS surface protein capture kit that we offer (Diagram 3.7). The percentage of reads that map to either apolipoproteins, fibronectin, IgG, or serum albumin when there's no post-isolation clean-up is extremely high. Over 45% of all the reads.

So if you're performing expensive mass spec where almost half of your reads are of background proteins, you may be missing out on very important biomarkers!

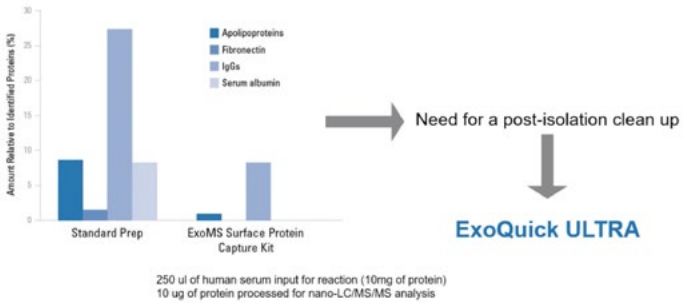
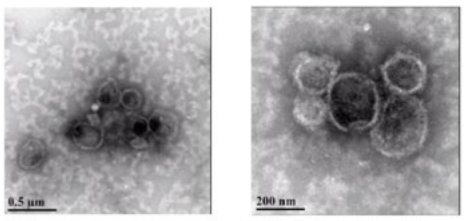


Diagram 3.7: Protein Background Detected by Mass Spec.
From SBI



Transmission electron micrographs of EVs isolated from human serum using ExoQuick ULTRA. The same sample is shown at two different magnifications. Multiple vesicles with typical EV morphology can be seen in each image

Image 3.1: EVs Isolated with ExoQuick ULTRA Display Typical EV Morphology.
Taken from powerpoint slide show. Please provide a citation.

ExoQuick ULTRA EV Isolation Kit

The need for a post-isolation clean-up is thus very clear. And that's exactly why we came up with ExoQuick ULTRA.

EVs isolated with ExoQuick ULTRA display a morphology you'd expect with EVs (Image 3.1).

With ExoQuick ULTRA you're also able to significantly reduce the amount of background and contaminating proteins, like albumin and IgG. Consequently, the detection of exosomal markers (e.g. CD9, CD81, and Hsp70) is significantly increased compared to other commercially available kits and ultracentrifugation (Diagram 3.8).

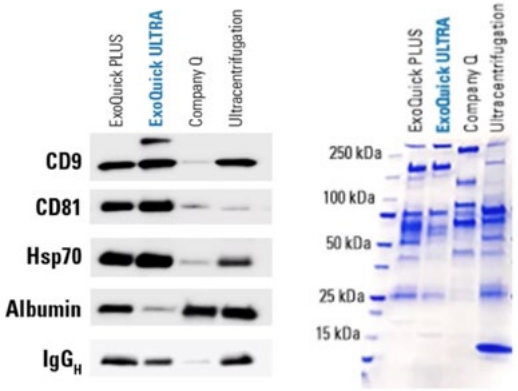


Diagram 3.8: Increased Purity with ExoQuick ULTRA.
A coomassie blue-stained protein gel comparing the protein content of exosome preps isolated using different methods shows only a few, defined protein bands in the ExoQuick ULTRA lane compared to the other methods. Western blotting of the gel shows that the ExoQuick ULTRA prep contains the highest levels of exosome-specific markers CD9, CD81, and Hsp70 and the lowest levels of the carryover proteins albumin and IgGH. In contrast, the prep from Company Q appears to be primarily albumin, and even the sample prepared using ultracentrifugation contains considerably higher levels of both albumin and IgGH. Each lane was loaded with 7 µg of total protein as measured using a fluorometric Qubit protein assay.
From SBI

This is because, as we remove albumin and IgG, we end up loading relatively higher amounts of EVs than with other EV preps.

ExoQuick ULTRA also allows you to increase your yield. This enables you to isolate more EVs per normalized input volume and per milligram of protein as compared to other commercially available kits or ultracentrifugation (Diagram 3.9).

How can you harness the power of ExoQuick ULTRA?

There's actually a very easy protocol you can follow (Diagram 3.10).

- Add ExoQuick ULTRA to 250 µL of serum or plasma and incubate for 30-minutes at 4oC
- Collect EVs by spinning at 3,000g for 10 minutes
- Resuspend EVs and add to pre-washed ExoQuick ULTRA columns
- Spin 1,000g x 30 sec and collect EVs—they are now ready to use

The main advantages of ExoQuick ULTRA are:

1. ExoQuick ULTRA results in cleaner preps
2. ExoQuick ULTRA increases the yield of EV isolation when starting with the same amount of material as compared to ultracentrifugation or other kits.

This allows for fast, cost-effective, and improved biomarker detection.

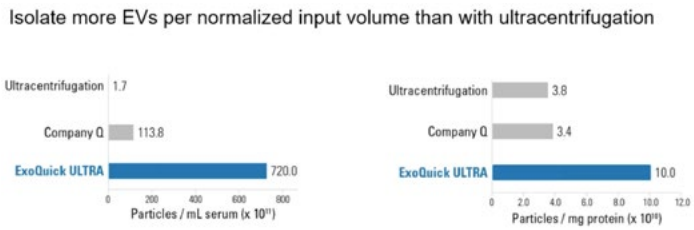


Diagram 3.9: Increased Purity with ExoQuick ULTRA Compared to Alternative Methods.
Fluorescent nanoparticle tracking analysis (fNTA) demonstrates the high EV yields delivered by ExoQuick ULTRA compared to ultracentrifugation. Comparison of different isolation methods on EV yields by both volume of input serum (per mL, A) and amount of input serum protein (per mg as measured by fluorometric Qubit protein assay, B). Particle number was measured using fNTA, a technique which specifically detects EVs. From SBI.

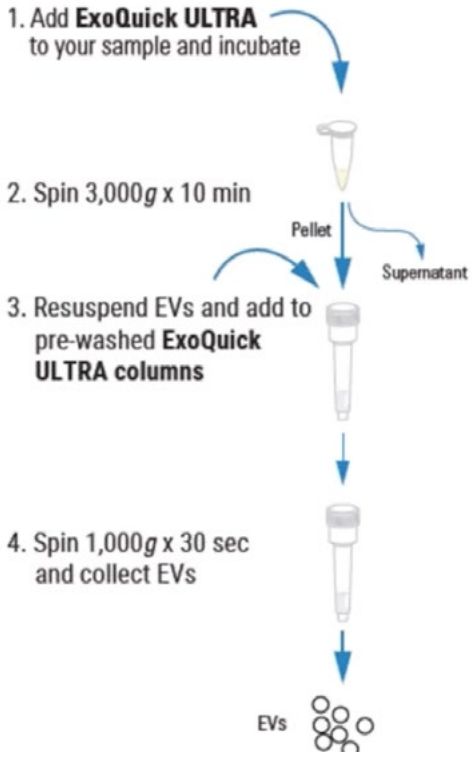


Diagram 3.10: ExoQuick ULTRA -How does it work?
From SBI.

Need High-Throughput? You Got It.

We believe that ExoQuick ULTRA is by far the market's best way of processing individual samples.

But what if you need an EV isolation system that's fast, easy, and high-throughput to boot? What if you need to quickly collect and compare EVs from multiple sources at once?

We've developed the industry's leading solution for that, too.

It's called the SmartSEC HT EV Isolation System; a proprietary chromatography-based EV isolation method that features an impressive, 96-well plate, format that allows you to isolate EVs from as many samples in less than 1 hour.

Most importantly, SmartSEC HT is a high performance system that achieves levels of purity and yield better than ultracentrifugation and comparable systems out on the market.

We have the data to back our claims too (Diagram 3.11 & Diagram 3.12).

As you can probably imagine at this point, the SmartSEC HT is a very powerful system that can accelerate and improve your EV biomarker discovery as well as diagnostic and therapeutic R&D.

And it can be employed with minimal fuss. Three easy steps, in fact (Diagram 3.13).

Step 1. All you need to do is prep the filter plate and add 250 – 500 µL of cleared serum or plasma into each well. Incubate thereafter.

Step 2. Centrifuge to elute the first fraction.

Step 3. Add an equal volume of SmartSEC Isolation Buffer and centrifuge again into a clean plate to elute the second fraction.

That's all there is to it.

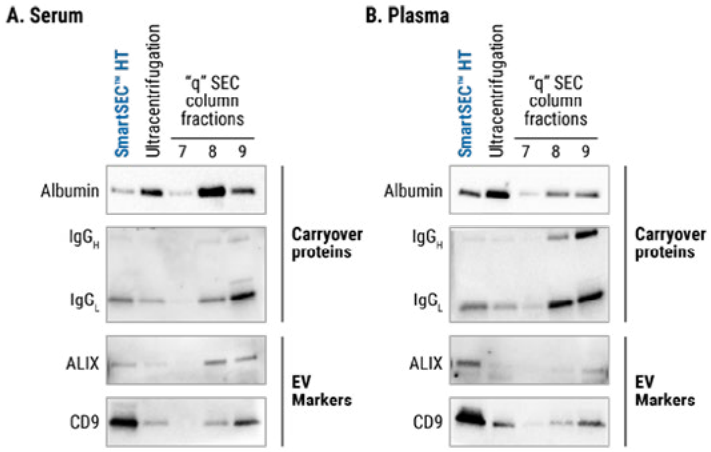


Diagram 3.11: SmartSEC™ HT delivers high yields of EVs with low amounts of undesirable carry-over protein

EVs were prepared from 500 µL of serum (A) or plasma (B) using the indicated methods. For Western blot analysis, 1 µg protein equivalent from the first fraction was loaded into each lane. SmartSEC™ HT performs better than ultracentrifugation and a competitor's "q" SEC column.

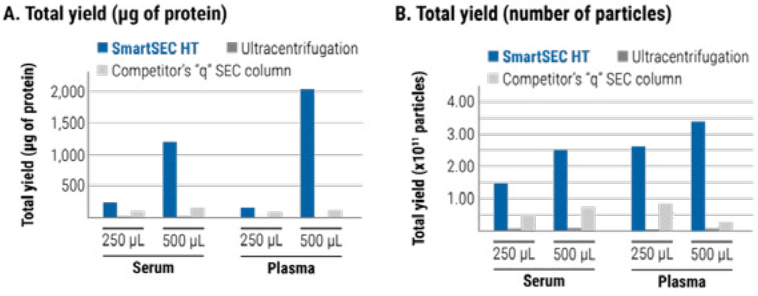


Diagram 3.12: SmartSEC™ HT delivers higher yields than ultracentrifugation and a competitor's "q" SEC column.

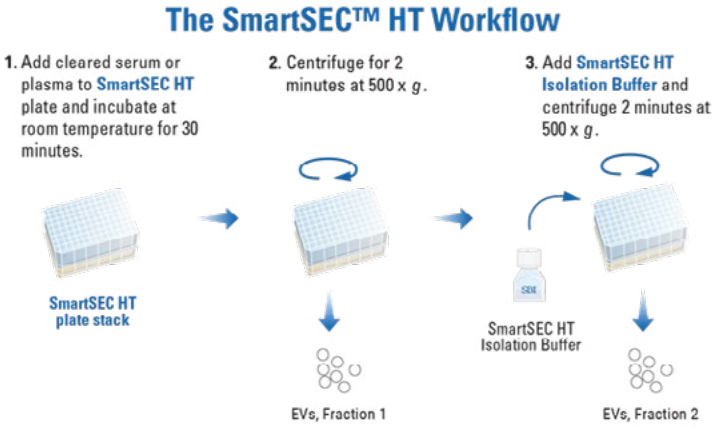


Diagram 3.13

ExoQuick Q&A

Q. Do EVs secrete nucleocapsids into the bloodstream?

A. Given the currently available literature on the point, we know that while nucleocapsids can be found inside of EVs, they aren't secreting them.

Q. What's the size of the vesicles that you can recover with ExoQuick ULTRA?

A. Depending on the prep, between 70-200 nm.

Q. What's the best way to isolate EVs from culture media?

A. This answer depends on the downstream applications you're going to use with your exosomes; this will alter your methodology. Importantly, we have a version of ExoQuick ULTRA that's specifically designed for tissue culture media and fluids that are less abundant, like CSF or urine. This version is called ExoQuick ULTRA TC.

Q. Can ExoQuick be used for functional EV isolation?

A. Yes, definitely. This is the express purpose of ExoQuick ULTRA.

Q. What would be the best system to use when trying to obtain enough small RNAs from EVs for library preps, for NGS?

A. ExoQuick ULTRA would be a very good choice in the sense that you're getting rid of background proteins, so you have a much cleaner prep while simultaneously increasing yield.

Q. How many milliliters are required for human serum?

A. You can start with 100-250 microliters.

Q. Does ExoQuick have a high throughput format, like a 96-well format for serum?

A. We don't have this yet. We understand that for clinical applications it makes things much easier, but actually, it is even better to tackle many samples with a precipitation-based approach than relying upon ultracentrifugation or other approaches.

Q. What is the range or heterogeneity of EV size in different samples?

A. The range of EV size is quite heterogenic as it includes exosomes and microvesicles that all vary in size from 70-250 nm. There's no specific cut-off for exosomes or microvesicles with respect to differences in size between the two.

Q. Do you treat the exosome prep with proteases before isolation in order to eliminate contaminating proteins?

A. No. In general, proteases won't eliminate the contaminating proteins and there's no good reason to add more proteins to your isolation procedure either.

Q. How would you dissolve an exosome pellet in PBS; as they can be difficult to dissolve?

A. While it may take 1-2 minutes of pipetting, even the hard-to-dissolve pellets will be able to dissolve.

Q. Do you recommend confirming EV markers with TM?

A. For general exosomal or EV markers, western blotting is just fine. In very particular applications, you may want to perform immunogold staining with TM with any specific biomarkers that you have identified.

Q. Are exosomes stable?

A. In terms of stability with respect to pH or proteases, they are very stable.

Q. Can ExoQuick ULTRA be used for the isolation of a specific size of EVs?

A. ExoQuick cannot discriminate between the different types of EVs. It isolates a heterogenic population of EVs.

Q. Where can I get the images found in this ebook?

A. If you want a copy of the images, please send us an email to **tech@systembio.com**