JWLudlow Consulting, LLC
EIN: 46-0753696
Mobile: 919-623-4221
Email: ludlow.johnw@gmail.com

White Paper

Concentration, size, and marker protein expression of exosomes isolated from amniotic fluid and umbilical cord blood

John W. Ludlow, Ph.D.

Executive Summary:

A single lot of exosomes isolated from amniotic fluid and umbilical cord blood, provided by the Center for Regenerative Medicine Laboratories, located at 1001 North east 125th street, Miami, FL. 33161, was analyzed in triplicate. The mean and mode sizes of the particles showed consistency within each the lot for the triplicate samples. Particle size for all samples is the size expected for exosomes. Similarly, particle concentration within each lot is consistent. To confirm that these particles are indeed exosomes, surface protein analysis for CD9, CD63, and CD81 was performed. The relative amounts of CD9, CD63, and CD81 showed consistency within each lot for the triplicate samples. The conclusion here is that the manufacturing process is robust and large quantities (trillions) of exosome particles are obtained per dosage unit of 1mL.

Introduction:

The term exosome is generally understood to reference a specific class of lipid-membrane bound extracellular vesicle (EV) characterized by a diameter of 40–150 nm and a density of 1.09–1.18 g/ml. Exosomes participate in a variety of cellular activities and have been shown to be isolatable from multiple body fluids including saliva, urine, plasma, serum, breast milk and amniotic fluid, as well as from the conditioned media of cultured cells (1, 2). In fact, exosomes can be isolated from any cell type which can be cultured. Purified exosomes have been demonstrated to have clinically relevant therapeutic bioactivity across multiple *in vitro* and *in vivo* models (3).

In this white paper, a single lot of exosomes isolated from amniotic fluid and a single lot isolated from umbilical cord blood, provided by the Center for Regenerative Medicine Laboratories, located at 1001 North east 125th street, Miami, FL. 33161, was each analyzed in triplicate. The purpose for this analysis was to determine the quantity of particles which may be isolated from a given dosage sample when processed by standard methods previously shown to isolate exosomes. Analysis of particle size and surface protein expression of these isolated particles was also undertaken to confirm that the particles were indeed exosomes.

Methods:

A 1mL sample of frozen amniotic fluid or umbilical cord blood was thawed and diluted to 12mL with sterile Dulbecco's phosphate buffered saline (DPBS) prior to centrifugation at 3000xg for 20min to pellet large microsomes and cellular debris. This clarified supernate was then centrifuged at 100,000xg for 2hr to pellet the exosome particles. The supernate was aspirated and discarded, and the resulting pellet resuspended in 1mL of sterile DPBS.

Particle quality was assessed using a Thermo NanoDrop spectrophotometer for protein determination and approximate RNA concentration by direct absorbance; exosome particles were not lysed, stained,

Mobile: 919-623-4221
Email: ludlow.johnw@gmail.com

or RNA extracted prior to taking these measurements. Particle diameter and concentration was measured by Nanoparticle Tracking Analysis (NTA) using a Particle Metrix ZetaView®.

Particle surface protein analysis was carried out using Milteny's MACSPlex Exosome Kit, human (Cat# 130-108-813). Particles are incubated with the antibody-coated MACSPlex Exosome Capture Beads, which bind to CD9, CD63, and CD81, which are proteins currently accepted in the exosome biology field to identify exosomes.

Results:

As shown in **Figure 1**., particles isolated from a single lot of umbilical cord blood (ALOCYTE) and a single lot of amniotic fluid (MATRIX) were each analyzed in triplicate (Vial 1, Vial 2, Vial 3). While the mean and mode sizes of the particles between the two lots show variation, there is consistency within each lot for the triplicate samples. In addition, particle size for all samples is in the size range (40-150nM) expected for exosomes. Similarly, particle concentration within each lot is consistent.

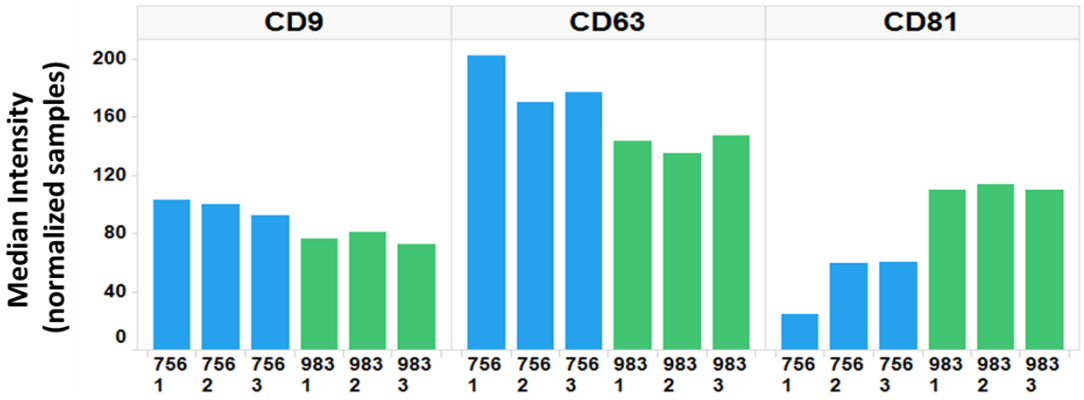
Figure 1.

Center for Regenerative Medicine Laboratories ZetaView Analysis

	Particle Diameter (nm)		Concentration
Sample	**Mean**	**Mode**	**(particles/mL)**
ALOCYTE_Vial1_ab2040756_062520B	112.8	72.5	6.30E+12
ALOCYTE_Vial2_ab2040756_062520B	104.2	77.5	5.40E+12
ALOCYTE_Vial3_ab2040756_062520B	112.9	87.5	4.40E+12
MATRIX_Vial1_ab2040983_06302020	134.2	92.5	6.30E+12
MATRIX_Vial2_ab2040983_06302020	133.9	92.5	6.10E+12
MATRIX_Vial3_ab2040983_06302020	137.1	87.5	7.00E+12

As shown in **Figure 2**., a single lot of particles isolated from umbilical cord blood (ALOCYTE) and a single lot of amniotic fluid (MATRIX) were each analyzed for surface proteins CD9, CD63, and CD81, in triplicate (Vial 1, Vial 2, Vial 3). While the relative amounts of CD9, CD63, and CD81 between the two lots show variation, there is consistency within each lot for the triplicate samples.

Figure 2.



Mobile: 919-623-4221
Email: ludlow.johnw@gmail.com

Conclusion:

The manufacturing process followed by the Center for Regenerative Medicine Laboratories is robust, resulting in large quantities (trillions) of exosome particles contained in a 1mL dose.

References:

1. Thery C, Clayton A, Amigorena S, et al. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Prot Cell Biol. 2006;3.22.1– 3.22.29. DOI:10.1002/0471143030.cb0322s30.

2. Whitford W, Ludlow JW, Cadwell JJS. Continuous production of exosomes. Gen Engin & Biotechnol New

3. Basu J, Ludlow JW. Exosomes for repair, regeneration and rejuvenation. Expert Opin Biol Ther. 2016;16(4):489-506. doi: 10.1517/14712598.2016.1131976. Review. PMID: 26817494.s 35 (16): 34, 2015.