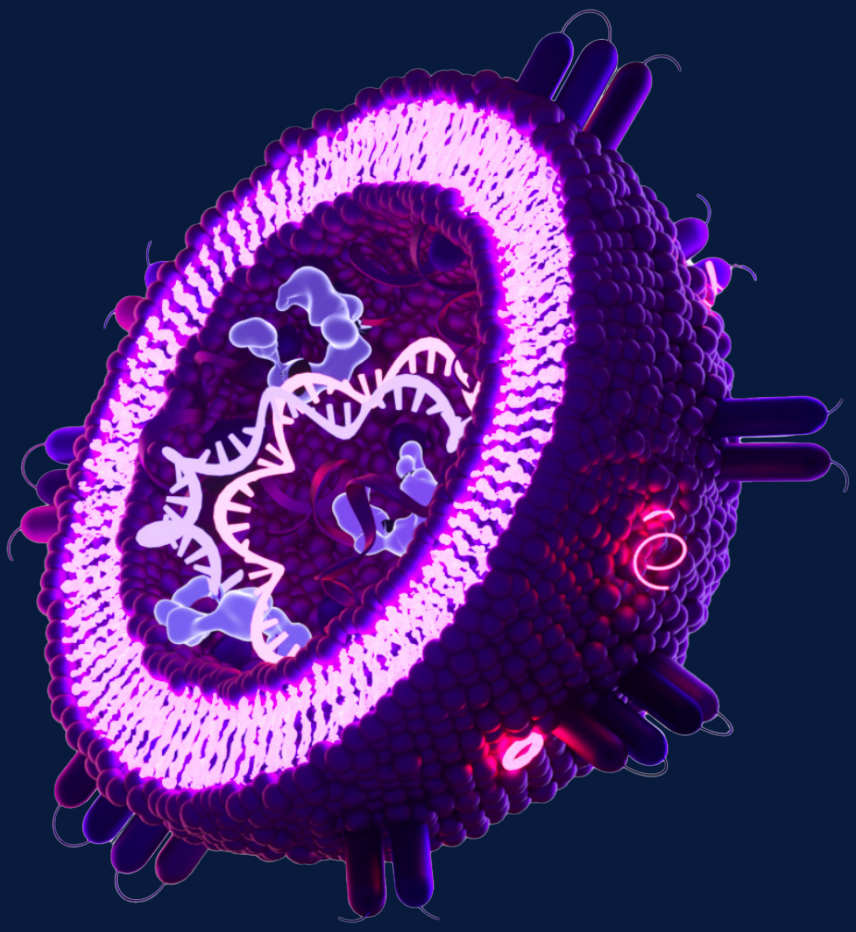
ISOLATING EVs FROM BLOOD PRODUCTS USING qEV COLUMNS



APPLICATION NOTE

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CONTENTS

1 INTRODUCTION

Extracellular vesicles (EVs) are membrane-bound particles produced by cells[1]. The term "EV" includes exosomes, microvesicles (sometimes called microparticles), oncosomes and other vesicles that are defined by their cellular origin, size, and surface markers[2]. EVs transmit signals between cells, and their ability to transport molecules to specific target cell populations make them attractive tools for diagnostic and therapeutic development[3].

Blood-derived EVs are a complex and heterogeneous population, comprising many EV types from multiple cellular origins. Blood-derived EVs are rich sources for biomarker discovery research, as many conditions and diseases can significantly impact the composition of circulating EVs. However, blood collection and processing can also be a cause of variability in EV isolates. This is largely due to platelets being activated during blood collection and processing, releasing abundant EVs *ex vivo* [4]. It is therefore important that sample collection and processing protocols:

- prevent platelet activation,
- remove platelets prior to storage and analysis, and
- are compatible with planned downstream analysis methods.

This application note provides general guidance, considerations, and recommendations on how to isolate EVs from blood products using Izon's qEV columns.

2 CONSIDERATIONS AND RECOMMENDATIONS

The detection and characterisation of blood-derived EV populations can often be confounded by artefacts that are generated during blood sample processing and EV isolation[5]. The collection process can alter the molecular integrity, function, and/or composition of biospecimens, so special considerations must be made when choosing the sample type (whole blood, plasma, or serum) that will be assessed as well as the blood collection and processing method that will be used. Some of these considerations have been outlined below and are represented in the flowchart in Figure 1.

2.1 Blood EV source

The EV source is an important consideration in the design of EV analytical studies. While some EV studies have been carried out using whole blood, it is extremely difficult to differentiate EVs from other particulate matter, so the number of applications is limited[5].

Plasma and serum are more commonly used than whole blood. However, serum can be contaminated with platelet-derived EVs that are produced during clot formation[5], which results in a sample that does not truly reflect the original EV composition and makes it difficult to obtain a sensitive analysis of less abundant EVs. For this reason, plasma is usually the preferred blood EV sample source.

2.2 Blood Sample Collection

Blood collection conditions can affect the quantity and characteristics of EV sub-populations present in blood samples. Collection variables such as storage temperature, transportation state, storage time, anticoagulant, and centrifugation protocol should be kept constant for all samples, as each of these can impact the number of EVs present in processed samples[5,6].

Factors to consider:

- Donor characteristics, including fasting status, can affect sample composition.
- Anticoagulants can affect downstream applications as well as sample composition, see Table 1.
- Platelets may be activated by the physical force associated with venepuncture; hence the first 2-3 mL of blood collected should be discarded[7].
- Haemolysed samples should be excluded[5].

Table 1: Anticoagulants Present in Blood Collection Tubes

ANTICOAGULANT	TUBE	MODE OF ACTION	COMMENTS
Trisodium Citrate	Blue Top	Prevents clotting by binding calcium	• Recommended by the International Society on Thrombosis and Haemostasis[5]
EDTA	Purple Top	Prevents clotting by binding calcium	• May activate platelets[6] • Can be suitable for RNA analysis[5]
Sodium Heparin	Green Top	Prevents clotting by inhibiting thrombin and thromboplastin	• Not generally recommended for EV studies • May activate platelets[6] • May block EV uptake by other cells[7] • Interferes with PCR reaction[5]
Acid Citrate Dextrose (ACD)	Yellow Top	Prevents clotting by binding calcium	• Affects osmotic balance of erythrocytes, which may lead to changes in EV composition/release[6] • Recommended for analysis of microparticles/microvesicles[8] • More efficient than citrate at inhibiting platelet EV release[5]

2.3 Blood Sample Processing

- After the blood has been collected, it is centrifuged to remove most circulating cells, like erythrocytes and leukocytes, as these cell types can release EVs into the blood after collection. It is important to limit the amount of time between collection and the first centrifugation step as much as possible; ideally this should be less than 30 minutes, but no more than 60 min[6].

- As platelets are smaller than other blood cells, they require specific conditions for their removal. We recommend reviewing the latest MISEV guidelines for best practice. If platelet-derived EVs are not of interest, platelet activation (release of EVs) must be carefully avoided whilst they are still present in the sample: cold can activate platelets, thus centrifuge samples at room temperatures[5]; shear can activate platelets, thus samples should be kept upright and avoid transport stress and agitation[5].

2.4 Pre-EV Isolation Storage of Plasma/Serum

Unless immediate plasma or serum-derived EV isolation and analysis is possible, short- or long-term sample storage will be required.

- All samples should be stored at the same temperature to limit effects on EV functionality.
- Storage at -80°C has shown the best results for maintaining EV numbers[6,9].
- Frozen samples should not be compared to fresh samples[6].
- Freeze thaw cycles should be kept to a minimum[5,6].

2.5 qEV Isolation

- There is a range of qEV isolation columns suited to different EV size ranges and sample volumes to match various research needs for blood-derived EVs.
- qEV Gen 2 columns are made with a proprietary agarose resin, which delivers a purer EV-containing eluate than standard SEC, including the discontinued qEV Legacy columns.
- qEV Gen 2 columns are available in the 20 nm, 35 nm, and 70 nm resin series. More information on qEV columns commonly used for blood EVs can be found in Table 2.

Table 2: Different qEV Gen 2 columns available to use with plasma/serum samples.

qEV COLUMN	INPUT qEV VOLUME*	PURIFIED COLLECTION VOLUME (PCV)**
qEVsingle	150 µL	680 µL
qEVoriginal	500 µL	1.6 mL
qEV1	1 mL	2.8 mL
qEV2	2 mL	8 mL
qEV10	10 mL	20 mL
qEV100	100 mL	200 mL

*qEV columns are optimised to maximise purity using human plasma samples.

**Default PCV specified for use of qEV Gen 2 columns on the Automatic Fraction Collector. The default recommended PCV provides a balance of EV recovery and high purity. Consult your qEV User Manual for more information on recommended parameters.

2.6 Process overview

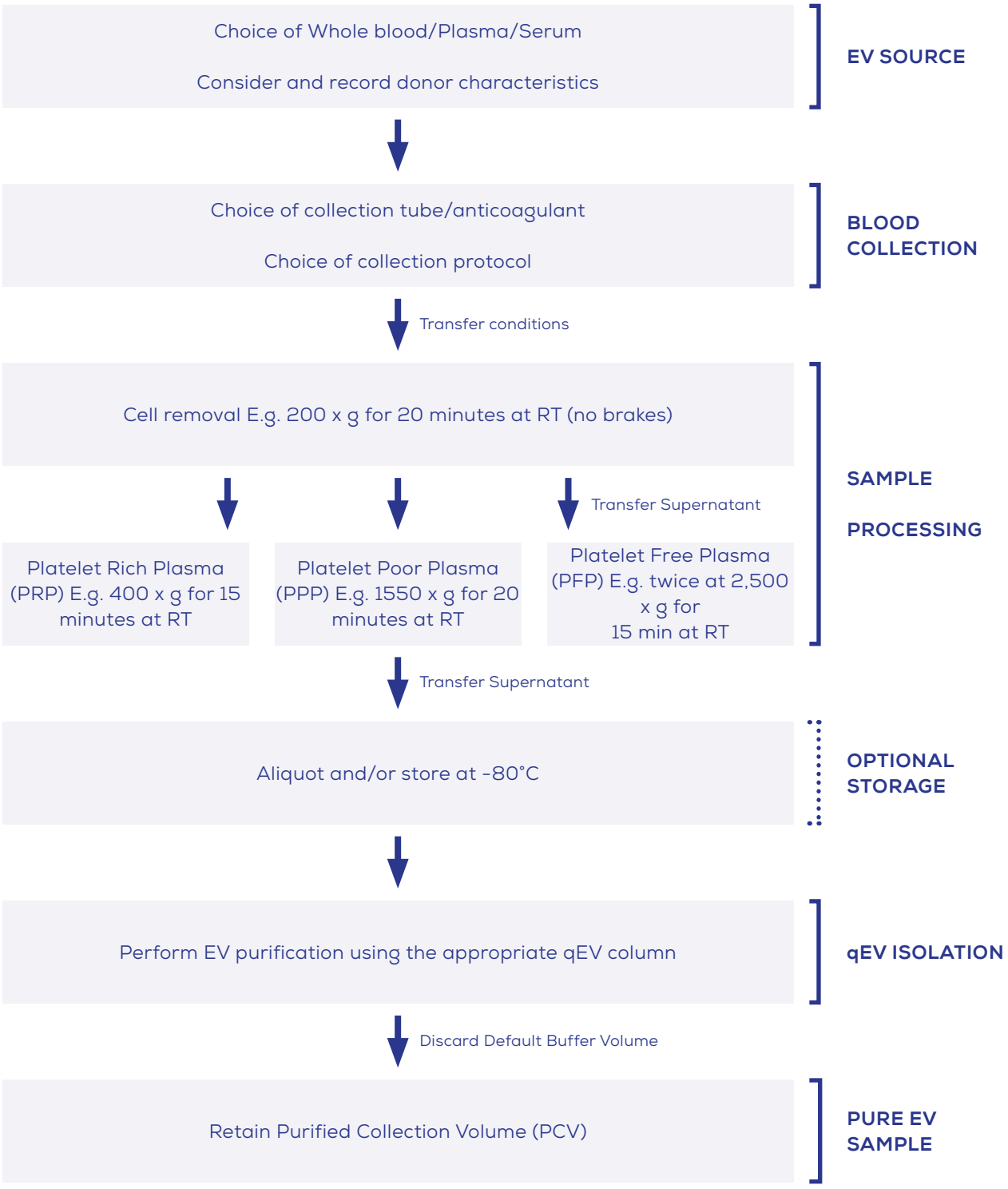

Figure 1: Flowchart summarising key considerations and stagess for the isolation of EVs from blood products using the qEV platform.

3 SUGGESTED MATERIALS

- Two evacuated blood collection tubes
- One ≥ 21-gauge needle
- Personal protective equipment
- Tourniquet
- Blood transfer device
- Puncture resistant sharps container
- Centrifuge with swing-out rotors capable of spinning at 2,500 g for 15 min.
- 1000 µL pipette
- 200 µL pipette
- Fresh 1 x PBS solution
- Sterile 0.22 µm syringe filter
- Sterile syringe
- Izon qEV column

4 METHODS

4.1 Example Protocol: Adult Blood Collection

1. Perform blood draw by a trained professional, in accordance with local medical regulations and ethical guidelines.
2. If possible, discard the first 2-3 mL of blood.
3. Collect required blood in chosen blood collection tube, considering that approximately 50% of blood volume corresponds to plasma.
4. Gently invert the tube 8-10 times to mix the blood with anticoagulant or components in the tube.
5. Allow the tubes to incubate at room temperature (20-24°C) for 10-20 min, but no more than 60 min.
6. Centrifuge tubes at 200 x g for 15-20 minutes at room temperature (RT)
    a. Make sure that the brake has been turned off.
    b. Use a centrifuge with a swing-out rotor as opposed to fixed rotors.
7. Transfer supernatant to a clean tube and centrifuge at appropriate speed and time to remove desired amount of platelets (refer to Figure 1).
8. Collect plasma/serum into a new tube using a suitable pipette, drawing from the top of the tube, and leave approximately 100 µL of plasma/serum at the bottom of the tube to avoid disturbing the pellet.
9. Store plasma/serum sample at -80 °C or liquid nitrogen.

4.2 Overview of qEV Isolation

See the relevant qEV User Manual and Safety Data Sheet for full details.

1. Prepare fresh 1 x PBS solution and filter using a sterile 0.22 µm syringe filter
2. Equilibrate and flush the qEV column with room-temperature PBS solution
    a. Degassed and room temperature buffers will help to avoid air bubbles forming in the gel bed.
3. Thaw plasma/serum sample and load an appropriate volume of sample onto the loading frit of the qEV column.
    a. Be sure that the volume of the sample is appropriate for the type of qEV column used (refer to Table 2 for information).
4. Begin collecting Default Buffer Volume and then Purified Collection Volume as directed in the qEV or AFC User Manual.
    a. Different samples may give slightly different elution profiles and purity, hence an initial measurement of EV concentration and protein contaminants in collected volumes is recommended.
5. After all volumes have been collected, flush the column with recommended volumes of 0.5 M sodium hydroxide (NaOH) followed by PBS buffer, found in each specific qEV User Manual.
    a. It is recommended to flush and refill the column with buffer containing a bacteriostatic agent (e.g., 0.05% sodium azide or 0.05% ProClin 200) prior to storage.
6. Store the column according to the instructions in the qEV User Manual.

5 REFERENCES

Izon Science publishes this method as a service to investigators. Detailed support for non-qEV aspects of this procedure might not be available from Izon Science.

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6 RESOURCES

For more information, application notes, technical bulletins, and user manuals, please visit Izon Support: support.izon.com

To order additional Izon reagents, please visit the Izon Store: store.izon.com

For Research Use only. Not for use in diagnostic procedures.

Note: Users are responsible for ensuring compliance with all relevant institutional, health and safety, and ethical regulations when collecting and handling human blood samples.

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