Navigating Obstacles in Exosome and Cell-Free RNA Extraction Protocols

Anna Sekyrova[1], Pavel Stejskal[1], Josef Srovnal[1], Marian Hajduch[1]

[1]Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc, Czech Republic

INTRODUCTION

Cell-free circulating RNA (cfRNA), particularly when encapsulated in exosomes, represents a promising avenue for minimally invasive cancer biomarker discovery. **Exosomes** (30–150 nm) carry a diverse array of RNA molecules that mirror the physiological and pathological states of their cells of origin, underscoring their potential in cancer diagnostics and prognostics. However, the translation of exosome-derived cfRNA into clinical practice is contingent upon the reproducibility and standardization of isolation and extraction techniques.

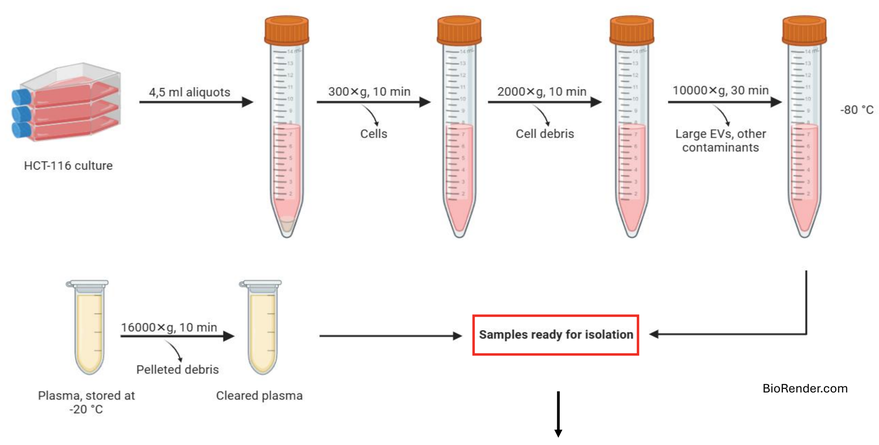
Aim

→ small EVs (preferentially exosomes) extraction and non-coding RNA extraction from within them

METHODS

Materials

cell culture-conditioned medium (CCM) from HCT-116 cells, 2 – 4 mL of **plasma** from healthy donors



Ultracentrifugation (UC) 100.000 x *g*, 90 min, 4 °C

- 38,5 mL open-top tubes
  - 1 x 90 min* — Beckman Coulter Optima XPN-100
  - 2 x 90 min* — SW 32 Ti rotor
- 1,5 mL UC tubes with adaptors* — 50.2 Ti Fixed-Angle rotor

Commercial kits

- Norgen ExtraClean Plasma/Serum Exosome Purification (part one) and RNA Isolation Kit
  - Part one (exosome purification)*
  - Complete protocol with RNA extraction
  - Complete protocol + DNase treatment
- Qiagen exoRNeasy
- Qiagen exoEasy*

→ ***RNA isolation**
Qiagen miRNeasy

→ **RNA quantification**
Agilent Bioanalyzer RNA 6000 Pico Kit

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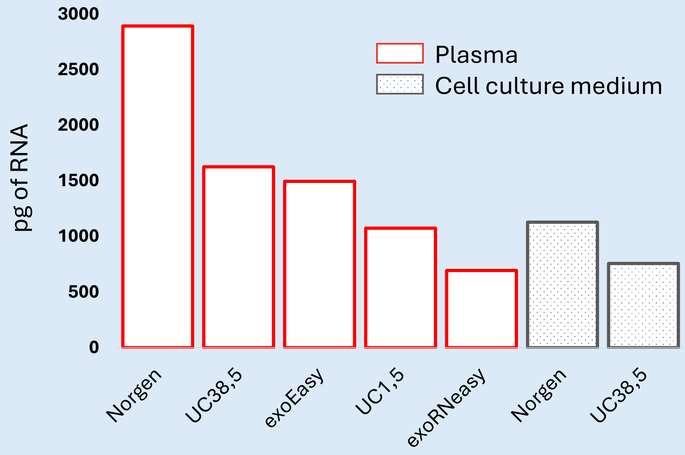
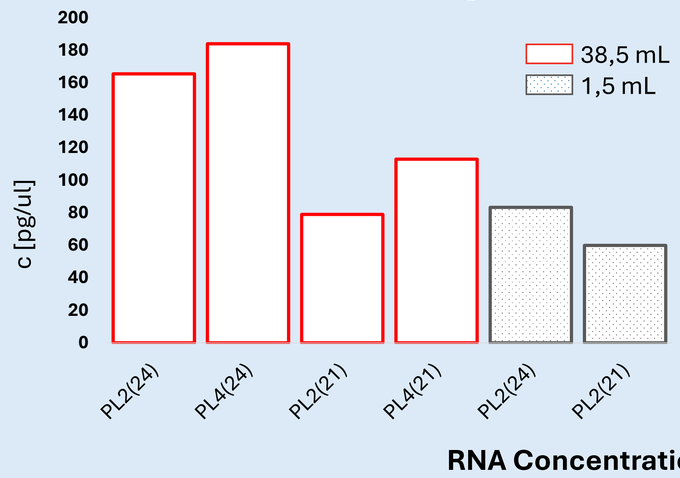
RESULTS

A **Average RNA Amount per mL of Sample**

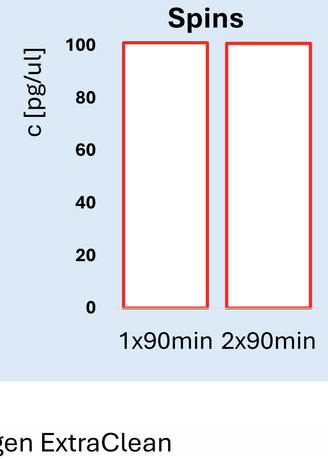


B **RNA Concentration After Ultracentrifugation**



(A) The Norgen kit yielded the highest RNA amount, nearly double that of the second-best method, UC in 38,5 mL open-top tubes, which performed comparably to the exoEasy kit. UC in 1,5 mL tubes ranked fourth, followed by exoRNeasy with the lowest yield, approximately one-third of the RNA amount compared to Norgen. Plasma samples consistently produced higher RNA yields over CCM samples. **(B)** Plasma samples again showed higher RNA yields than CCM samples. Samples derived from 4 mL plasma had higher yields than those from 2 mL plasma, though not proportionally double, with 2 mL still providing relatively good yields. The most significant factor affecting yield was sample age; plasma samples stored for 4 years approximately half the RNA concentration compared to more year old samples. **(C)** Results show no difference between single and double spin conditions, indicating that an additional spin with washing did not increase RNA yield.

C **RNA Concentration After Single vs. Double Ultracentrifugation Spins**



Norgen ExtraClean Plasma/Serum Exosome Purification and RNA Isolation Kit

- Inconsistent results
- Good RNA amounts (larger elution volume), but poor quality
- Great losses with DNase treatment

Qiagen exoEasy

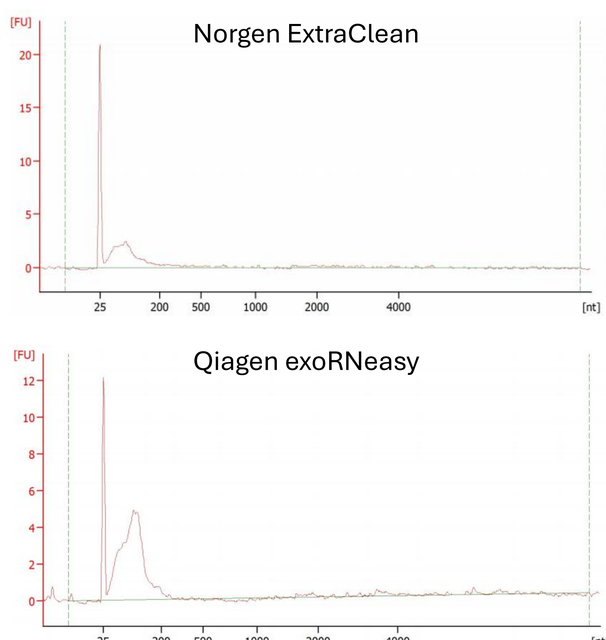
- Mainly short fragments

Qiagen exoRNeasy

- Also yields short fragments

Bioanalyzer as a tool for RNA quality assessment

- Not suitable as RIN (RNA integrity number) algorithm depends on intact 18S and 28S rRNA peaks
- rRNA usually absent or depleted in EV RNA



RNA Electropherogram of Norgen ExtraClean and Qiagen exoRNeasy kits Isolated RNA (Agilent Bioanalyzer)

CONCLUSION & FUTURE PERSPECTIVES

- **Isolation method critical:** Norgen ExtraClean highest yield, but significant losses with DNase treatment
- **Qiagen exoEasy/exoRNeasy:** predominantly enrich short RNA fragments, limiting full-length RNA applications
- **Sample type:** plasma yields superior to CCM; larger plasma volumes improve yield but not proportionally
  Sample age fundamental : older samples show significantly reduced RNA concentrations
- **Ultracentrifugation:** 38,5 mL tubes overall better, extra spin + wash do not improve yield
- **Bioanalyzer:** not the optimal tool for EV RNA quality assessment (no RIN) due to lack of rRNA peaks

Next steps

SEC and **magnetic immunocapture** for higher purity/yield + **PCR**-based quantification to improve sensitivity

References

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LET'S CONNECT
anna.sekyrova02@upol.cz

INSTITUTE OF MOLECULAR AND TRANSLATIONAL MEDICINE
NiCR
Funded by the European Union NextGenerationEU
CZECH RECOVERY PLAN
MINISTRY OF EDUCATION, YOUTH AND SPORTS