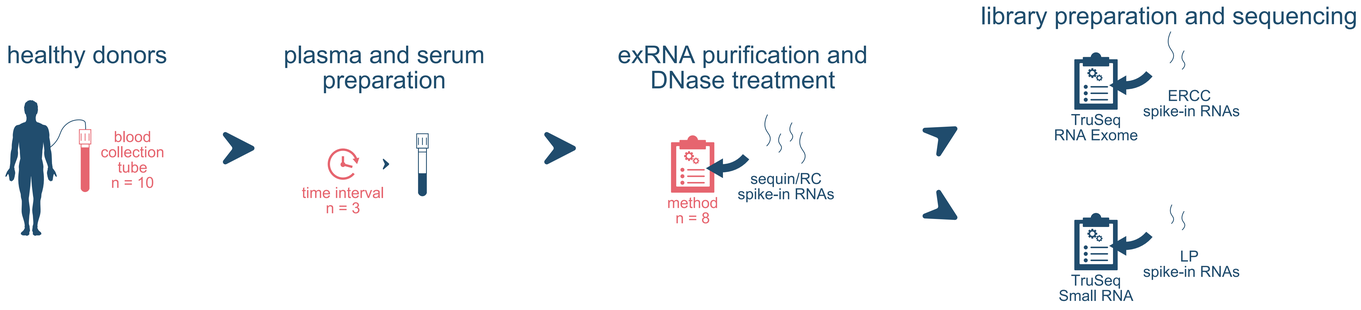
Substantial performance differences among RNA purification kits and blood collection tubes in the Extracellular RNA Quality Control study - important considerations for liquid biopsies

exRNAQC Consortium. CONCEPTUALIZATION: Decock A[†1], De Wever O[2], Everaert C[1], Helsmoortel HH[1], Hendrix A[2], Mestdagh P[3], Morlion A[1], Vandesompele J[3], Van Paemel R[4]; DATA CURATION: Avila Cobos F[1], Decock A, Everaert C, Helsmoortel HH, Morlion A, Van Paemel R; FORMAL ANALYSIS: Avila Cobos F, Everaert C, Fierro C[5], Mestdagh P, Morlion A, Vandesompele J, Van Paemel R; FUNDING ACQUISITION: Decock A, Mestdagh P, Vandesompele J; INVESTIGATION: Decock A, Deleu J[1], Dhondt B[6], Helsmoortel HH, Hulstaert E[7], Nijs N[5], Nuytens J[1], Philippron A[8], Schoofs K[1], Vanden Eynde E[1], Van Paemel R, Verniers K[1], Yigit N[1]; METHODOLOGY: Avila Cobos F, Decock A, Dhondt B, Everaert C, Fierro C, Helsmoortel HH, Mestdagh P, Morlion A, Nijs N, Philippron A, Vandesompele J, Van Paemel R; PROJECT ADMINISTRATION: Decock A; RESOURCES: Dhondt B, Hulstaert E, Kuersten S[9], Philippron A, Schroth G[9], Van Paemel R; SOFTWARE: Anckaert J[1], Avila Cobos F, Everaert C, Morlion A, Van Paemel R; SUPERVISION: Mestdagh P, Vandesompele J; VISUALIZATION: Avila Cobos F, Everaert C, Morlion A, Van Paemel R; WRITING - ORIGINAL DRAFT: Decock A; WRITING - REVIEW & EDITING: Avila Cobos F, Everaert C, Helsmoortel HH, Hulstaert E, Mestdagh P, Vandesompele J, Van Paemel R.

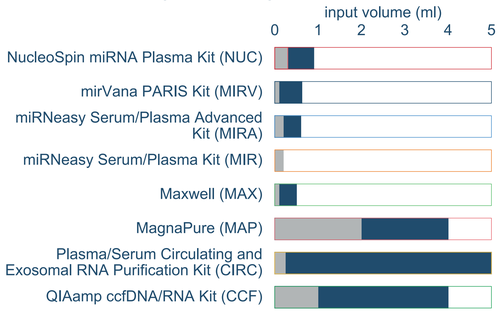
a.decock@ugent.be

How do pre-analytical factors affect downstream sequencing of blood-derived extracellular RNA (exRNA)?

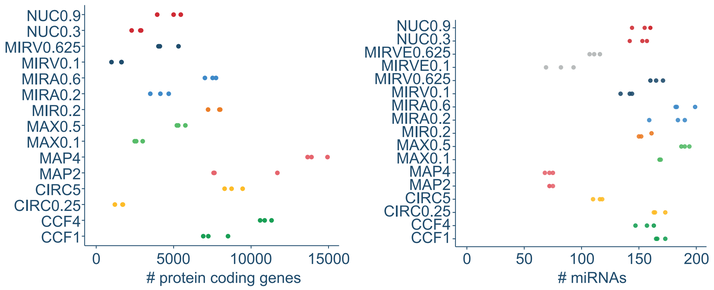


exRNA purification method

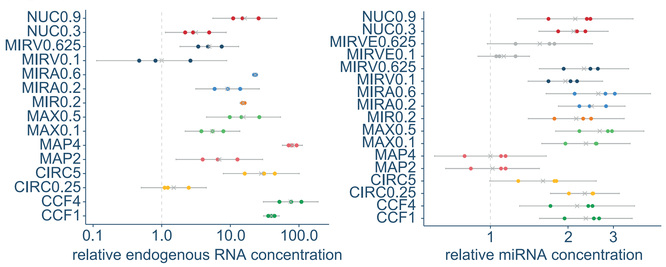
8 RNA purification methods are compared, testing the minimum and maximum recommended plasma input volumes.



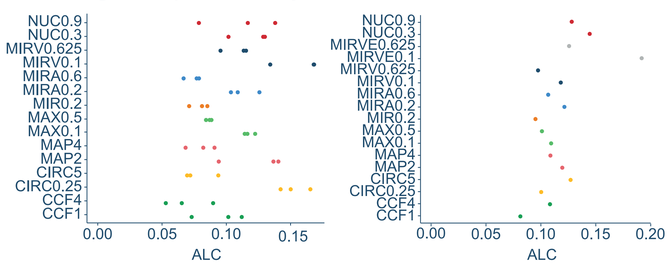
A high variability in transcriptome complexity is observed. Note that for profiling small RNAs using MIRV, also an alternative purification protocol was tested (i.e. MIRVE).



The relative endogenous RNA and miRNA concentration in the eluate varies considerably among purification methods. Shown are the ratios of endogenous RNA to ERCC spike-in RNAs (left) or miRNA to LP spike-in RNAs (right) as a measure for concentration.

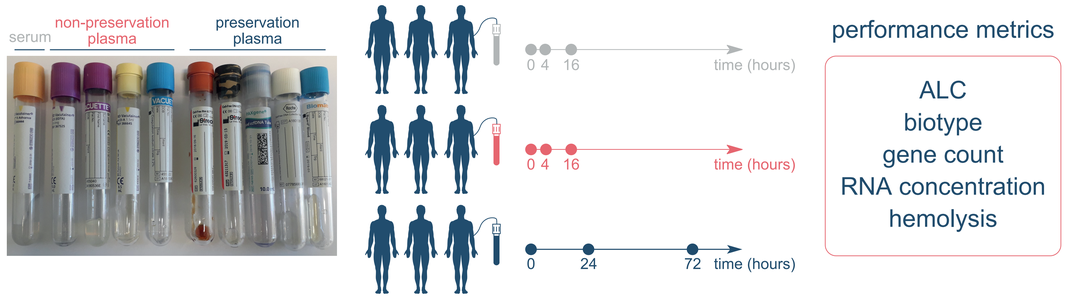


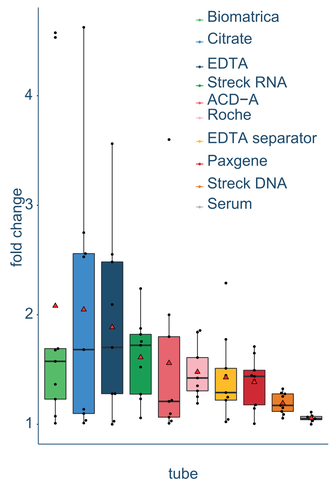
Large differences in reproducibility are observed. Shown are the areas left of the cumulative distribution curves (ALCs), comparing RNA purification replicates. Lower ALC-values indicate less deviations between replicates and hence better/higher reproducibility.

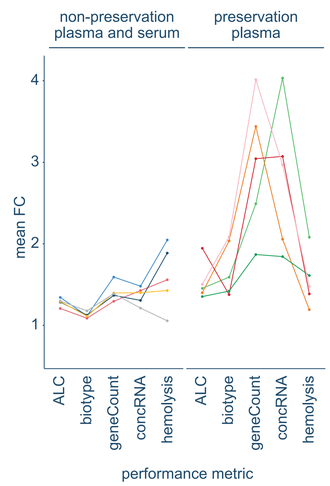


Bood collection tube - time interval between blood draw and plasma/serum preparation

Liquid biopsies are prepared from 10 different blood collection tubes at 3 different time points upon blood draw, profiled and evaluated using 5 performance metrics







The performance of preservation tubes for profiling mRNAs is generally compromised. For each donor and each tube, the fold change (FC) of each of the performance metrics between the different time intervals is calculated (left: distribution of the FCs for hemolysis; right: mean FC for each of the performance metrics and tubes). Lower FCs indicate a higher tube stability over time.

Conclusions and future perspectives

In the exRNAQC study, we comprehensively assess the impact of pre-analytical variables on deep transcriptome profiling of small and messenger RNAs. We provide robust quality control metrics for exRNA quantification methods with validated SOPs for sample collection, processing and profiling. Furthermore, we show substantial differences in terms of transcriptome complexity, exRNA concentration and reproducibility for the tested RNA purification methods and blood collection tubes. Based on these data we will now put forward a selection of pre-analytics to be further evaluated. This is paramount ground work for any future RNA-based liquid biopsy-guided precision oncology study.

Affiliations. [†]Presenting author. [1]Center for Medical Genetics, Ghent University, Cancer Research Institute Ghent (CRIG), Ghent, Belgium. [2]Laboratory of Experimental Cancer Research, Ghent University, Cancer Research Institute Ghent (CRIG), Ghent, Belgium. [3]Center for Medical Genetics, Ghent University, Cancer Research Institute Ghent (CRIG), Biogazelle, Ghent, Belgium. [4]Center for Medical Genetics, Ghent University, Department of Pediatrics, Ghent University Hospital, Cancer Research Institute Ghent (CRIG), Ghent, Belgium. [5]Biogazelle, Zwijnaarde, Belgium. [6]Laboratory of Experimental Cancer Research, Ghent University, Department of Urology, Ghent University Hospital, Cancer Research Institute Ghent (CRIG), Ghent, Belgium. [7]Center for Medical Genetics, Ghent University, Department of Dermatology, Ghent University Hospital, Cancer Research Institute Ghent (CRIG), Ghent, Belgium. [8]Lab for Experimental Surgery, Ghent University, Department of Gastrointestinal Surgery, Ghent University Hospital, Cancer Research Institute Ghent (CRIG), Ghent, Belgium. [9]Illumina Inc., San Diego, California, USA.