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From sample to treatment
Preclinical Factors

Rikke Fredslund Andersen
Biochemistry and Immunology
University Hospital of Southern Denmark, Vejle
rikke.fredslund.andersen@rsyd.dk

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Variation

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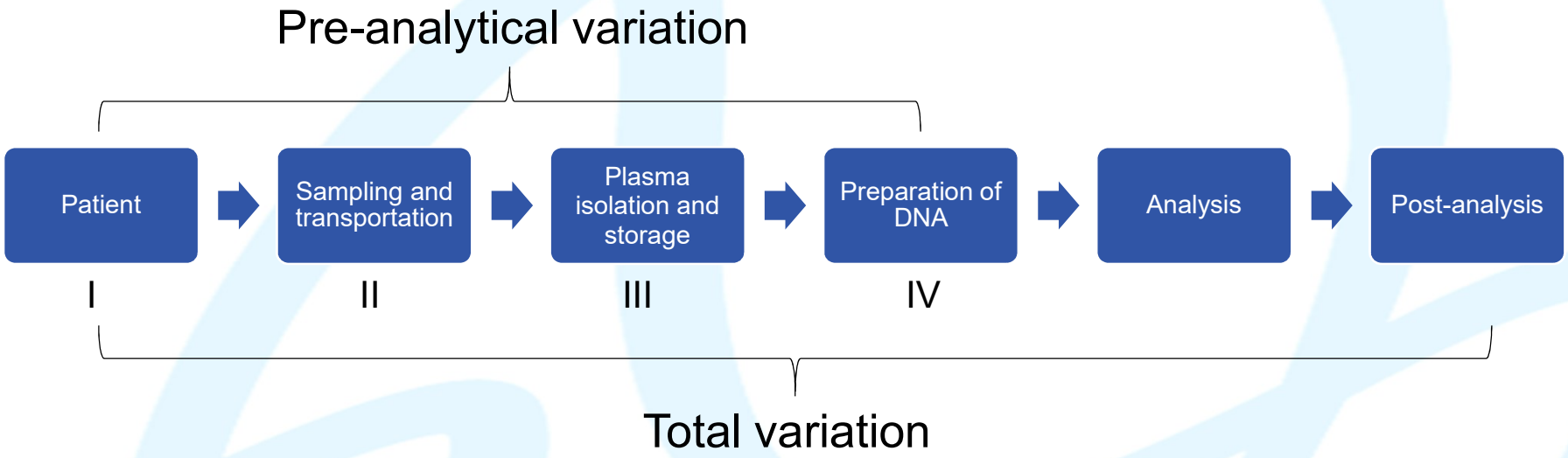

I. Biological variation, co-morbidities, physical activity, fasting, timing in relation to medical/surgical treatment
II. Collection tube (type/volume), syringe, stasis, first/second tube, agitation
III. Time to centrifugation, centrifugation, temperature, pipetting, storage conditions, freeze/thaw
IV. Extraction of DNA, (pre-amplification, denaturation, bisulphite conversion,…)

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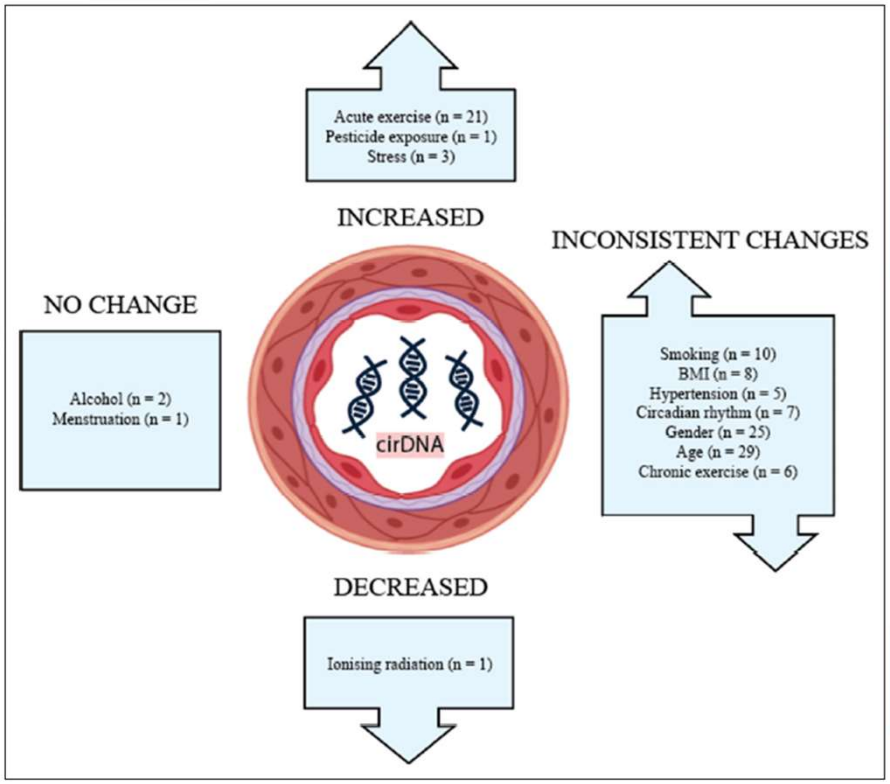

Yuwono *et al.* eLife 2021;0:e69679.

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Surgical treatment

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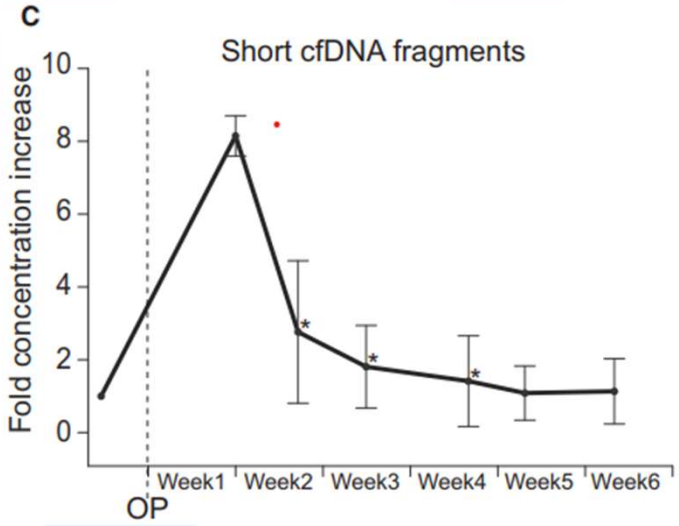
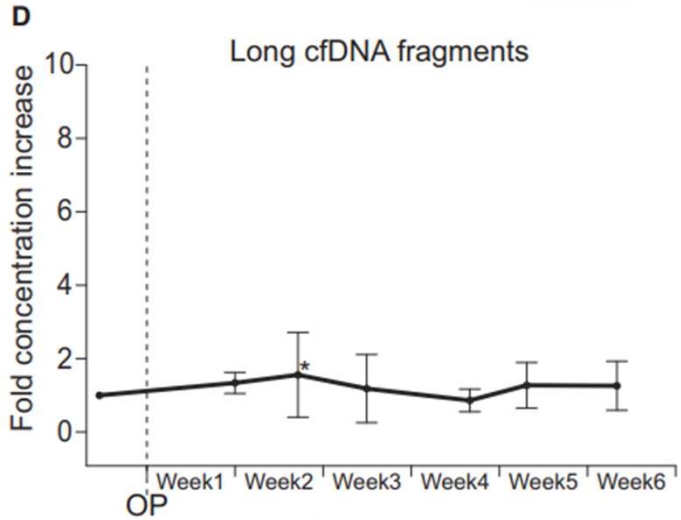
The effect of surgical trauma on circulating free DNA levels in cancer patients—implications for studies of circulating tumor DNA

Tenna V. Henriksen[1], Thomas Reinert[1], Emil Christensen[1], Himanshu Sethi[2], Karin Birkenkamp-Demtröder[1], Mikail Gögenur[3], Ismail Gögenur[3], Bernhard G. Zimmermann[2], The IMPROVE Study Group[†], Lars Dyrskjøt[1] and Claus L. Andersen[1]

1 Department of Molecular Medicine, Aarhus University Hospital, Aarhus N, Denmark
2 Natera Inc., San Carlos, CA, USA
3 Center for Surgical Sciences, Zealand University Hospital, Køge, Denmark

Molecular Oncology **14** (2020) 1670–1679





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Medical treatment

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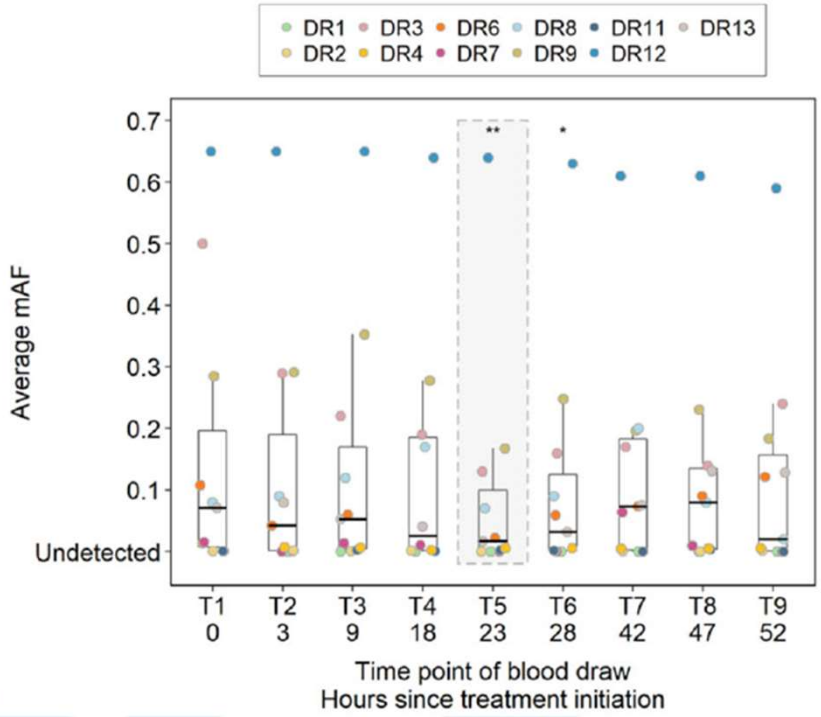

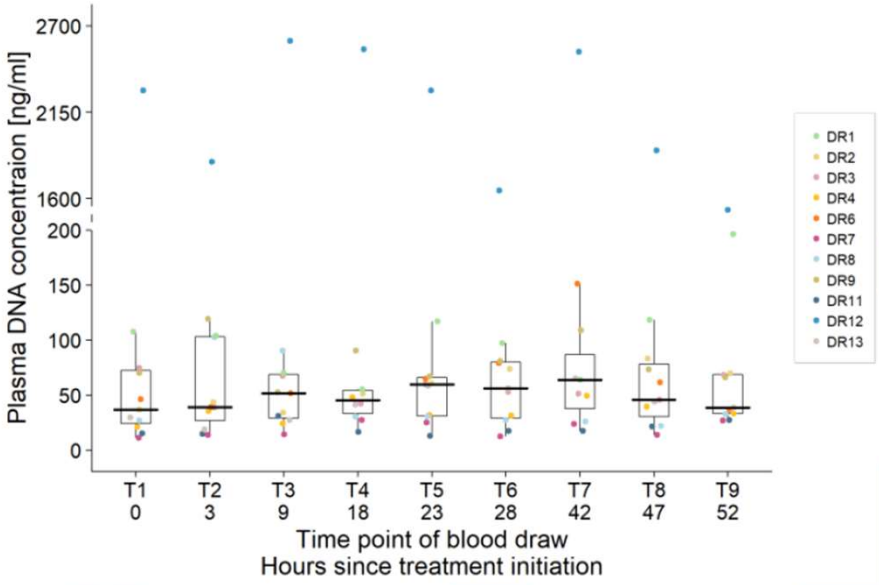

On-treatment measurements of circulating tumor DNA during FOLFOX therapy in patients with colorectal cancer

Tina Moser[1,10], Julie Waldispuehl-Geigl[1,10], Jelena Belic[1,9], Sabrina Weber[1], Qing Zhou[1], Samantha O. Hasenleithner[1], Ricarda Graf[1], Jasmin Alia Terzic[2], Florian Posch[2], Heinz Sill[3], Sigurd Lax[4], Karl Kashofer[5], Gerald Hoefler[5], Helmut Schoellnast[6], Ellen Heitzer[1,7,8], Jochen B. Geigl[1], Thomas Bauernhofer[2]✉ and Michael R. Speicher[1,7]✉

npj Precision Oncology 4:30 (2020)

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Variation

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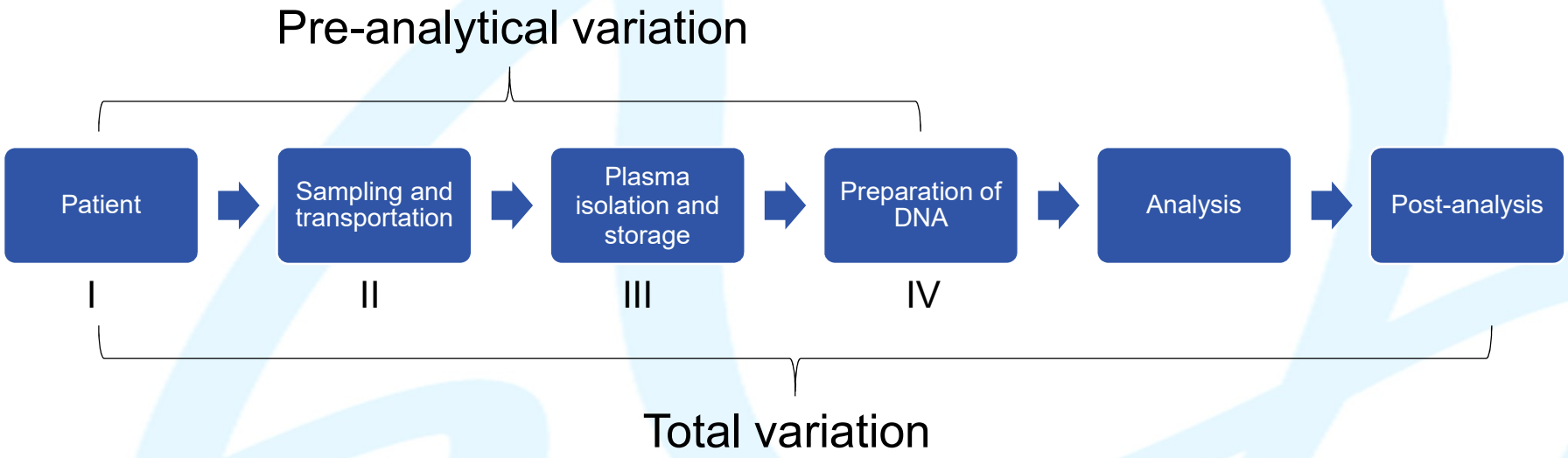

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EDTA vs. Streck tubes

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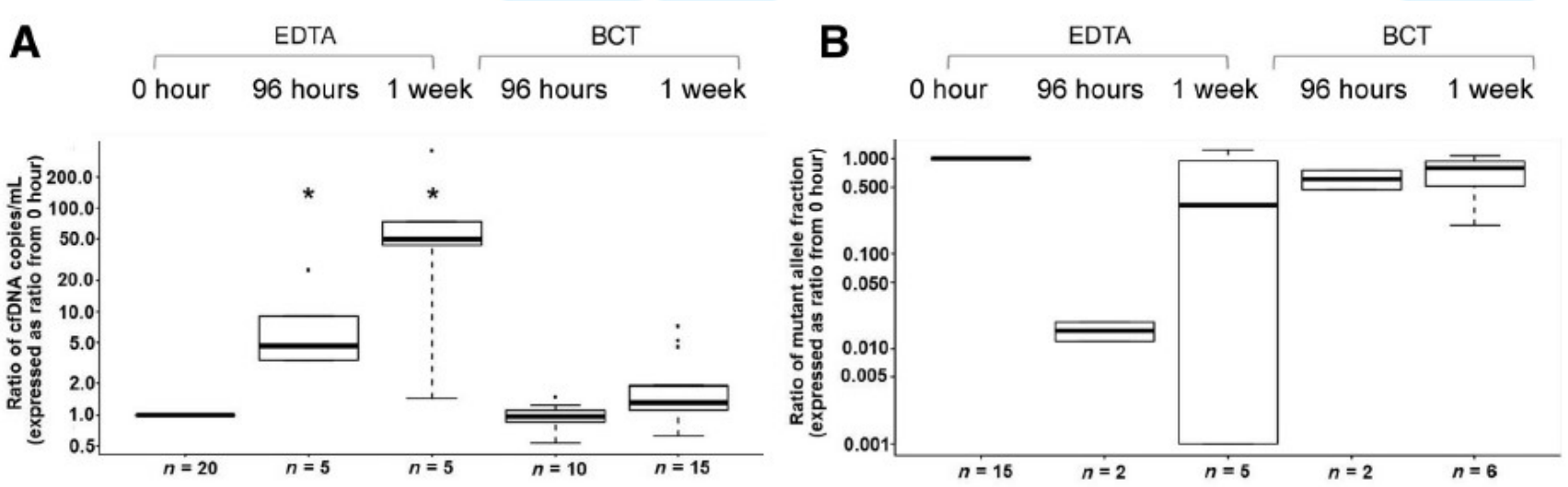

Effects of Collection and Processing Procedures on Plasma Circulating Cell-Free DNA from Cancer Patients

Check for updates

Bente Risberg,*† Dana W.Y. Tsui,*§ Heather Biggs,¶ Andrea Ruiz-Valdepenas Martin de Almagro,*§ Sarah-Jane Dawson,*¶‖ Charlotte Hodgkin,‖ Linda Jones,¶ Christine Parkinson,‖ Anna Piskorz,*§ Francesco Marass,*§ Dineika Chandrananda,*§ Elizabeth Moore,*§ James Morris,*§ Vincent Plagnol,** Nitzan Rosenfeld,*§ Carlos Caldas,*§¶‖ James D. Brenton,*§‖ and Davina Gale*§

The Journal of Molecular Diagnostics, Vol. 20, No. 6, November 2018

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Volume of blood

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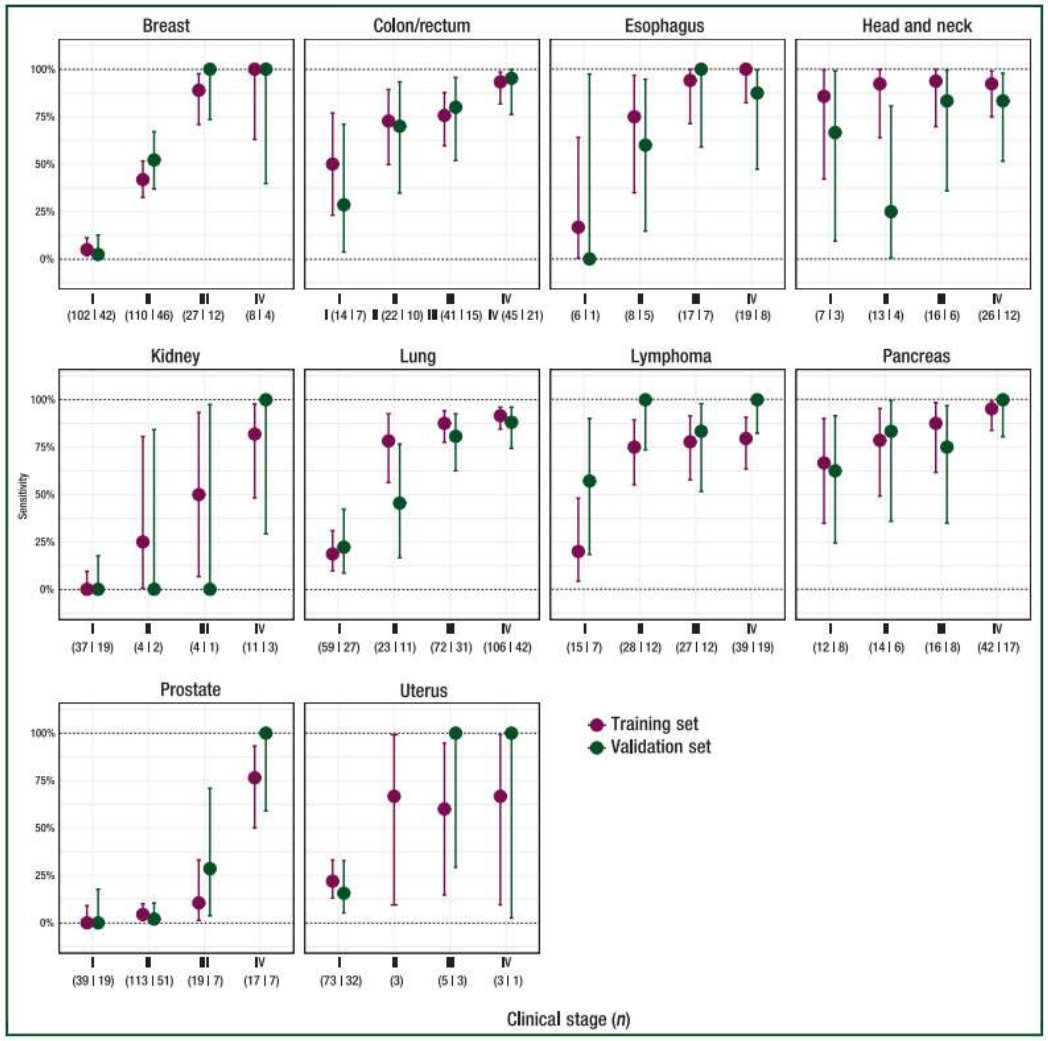
- Organ
- Stage
- Metastatic site



Liu MC *et al.*, Ann Onc 2020

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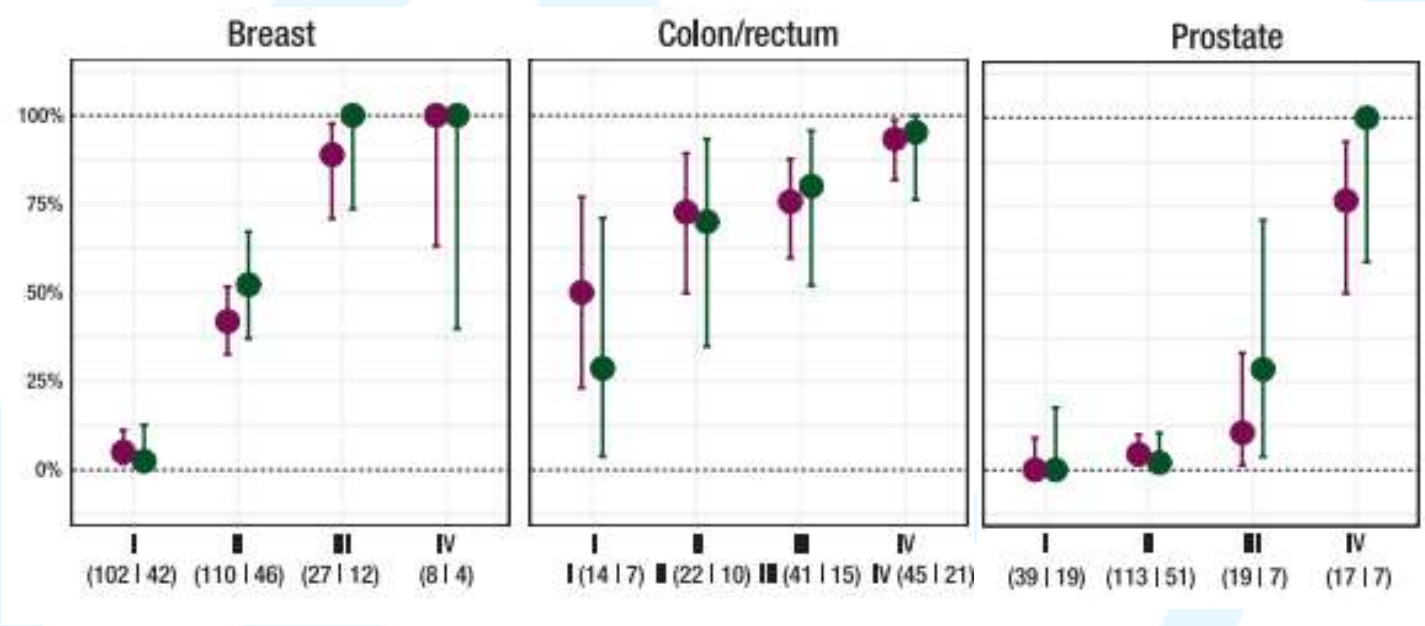
Organ and stage



Liu MC *et al.*, Ann Onc 2020

Metastatic site - CRC

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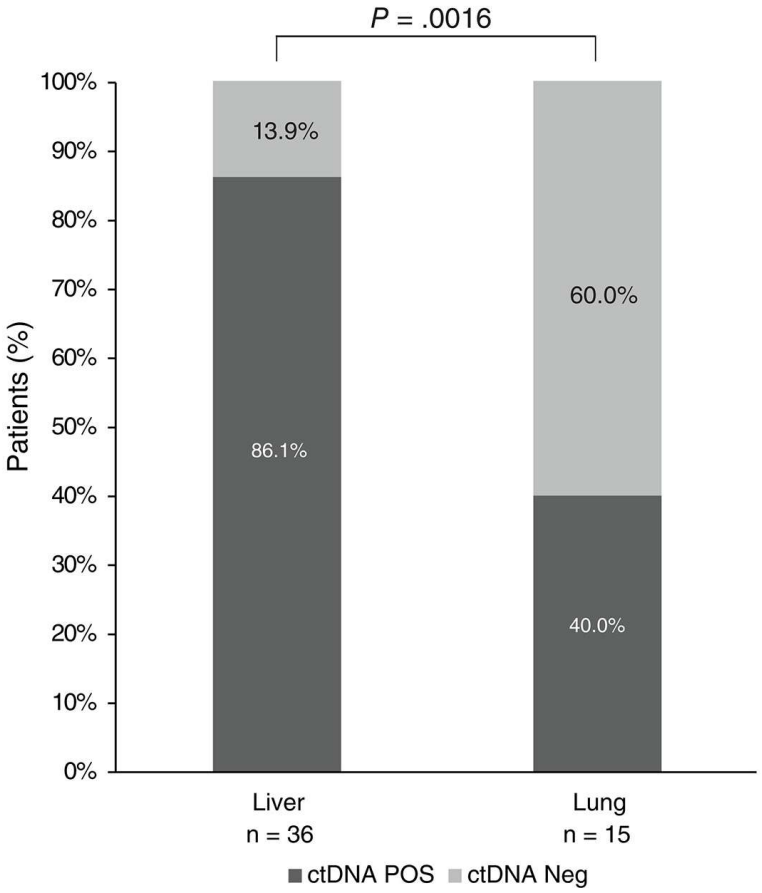

Reinert T et al, Intl Journal of Cancer, Volume: 150, Issue: 9, Pages: 1537-1548,
First published: 07 January 2022, DOI: (10.1002/ijc.33924)

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Variation

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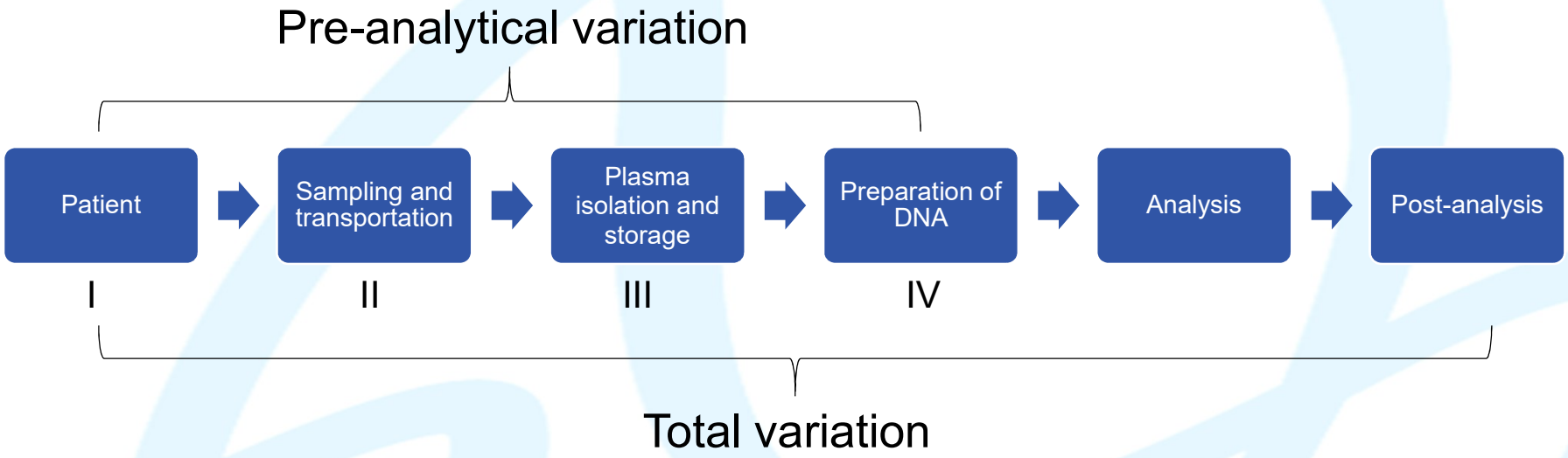

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Blood sampling

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- Tube type (EDTA, Streck, Paxgene)
- Processing (centrifugation (single/double), timing, pipetting)
- Storage of plasma / purified cfDNA (tubes, duration, freeze/thaw cycles)

ISO 20186-3:2019
Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 3: Isolated circulating cell free DNA from plasma

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ISO20186-3

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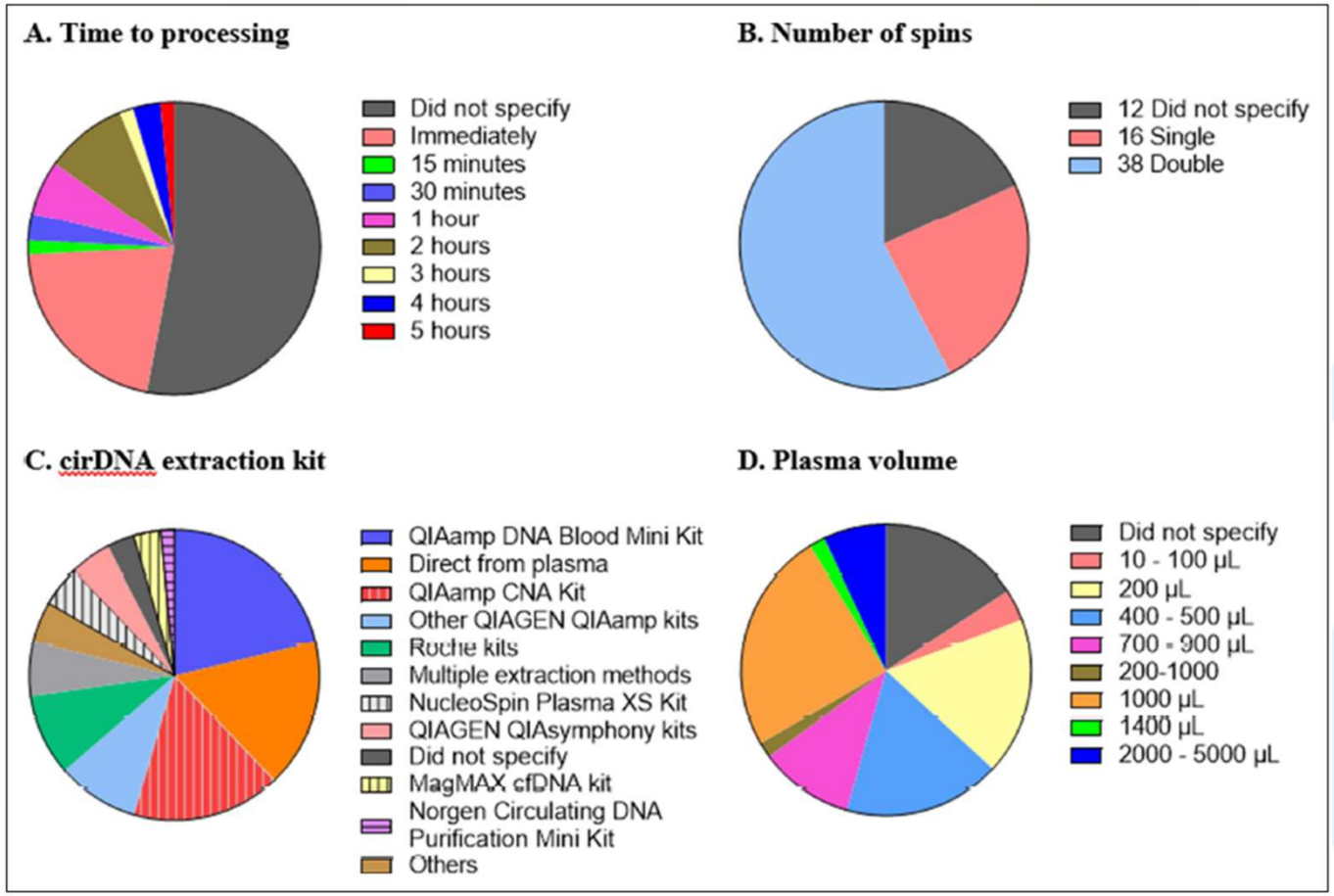
Yuwono *et al*. eLife 2021;0:e69679.



Time to centrifugation

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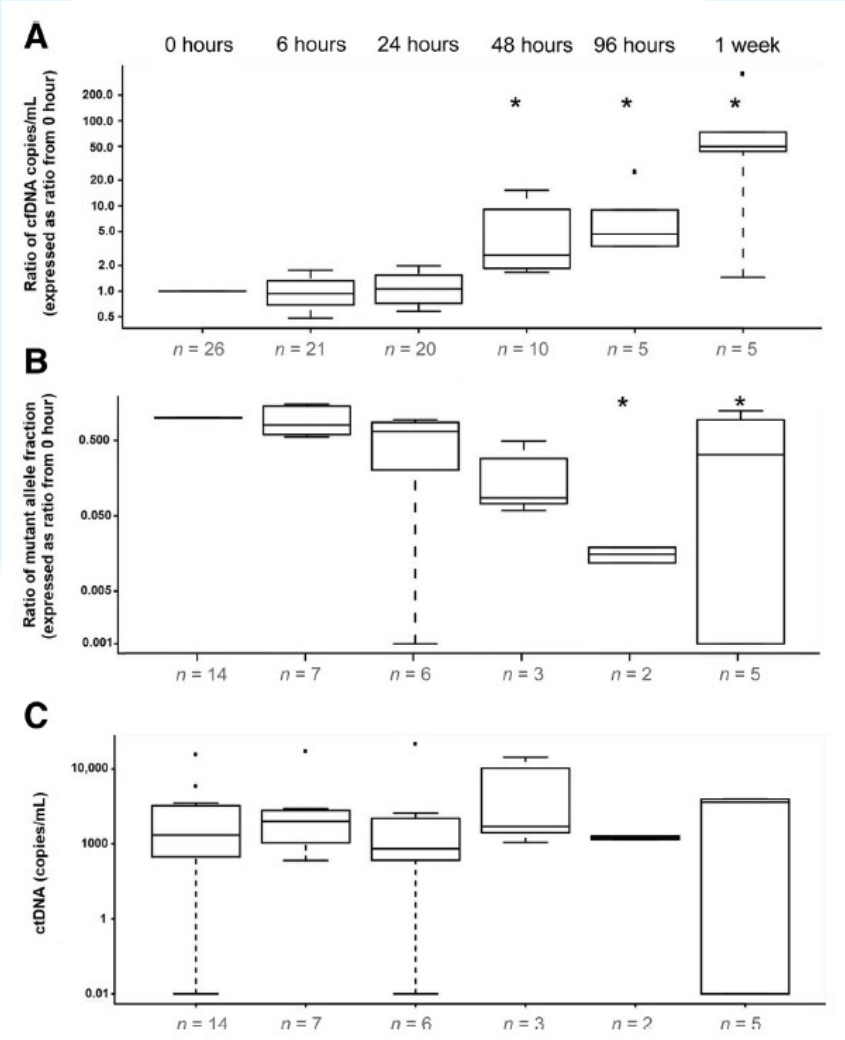
Effects of Collection and Processing Procedures on Plasma Circulating Cell-Free DNA from Cancer Patients

Bente Risberg,*† Dana W.Y. Tsui,*§ Heather Biggs,¶ Andrea Ruiz-Valdepenas Martin de Almagro,*§ Sarah-Jane Dawson,*¶‖ Charlotte Hodgkin,‖ Linda Jones,¶ Christine Parkinson,‖ Anna Piskorz,*§ Francesco Marass,*§ Dineika Chandrananda,*§ Elizabeth Moore,*§ James Morris,*§ Vincent Plagnol,** Nitzan Rosenfeld,*§ Carlos Caldas,*§¶‖ James D. Brenton,*‖ and Davina Gale*§

The Journal of Molecular Diagnostics, Vol. 20, No. 6, November 2018



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Recommendations

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- 1. centrifugation @ 1,600-2,500 g, 10 minutes
- Transfer supernatant to clean tube. Leave 0.5 cm above the buffycoat
- 2. centrifugation @ >10,000 g, 10 minutes
- Transfer supernatant to clean tube. Leave 0.5 cm above the pellet
- Proces within 2-4 hours if using EDTA tubes
- Long-term storage @ -80^{o}C
- Freeze-thaw 1-2 times

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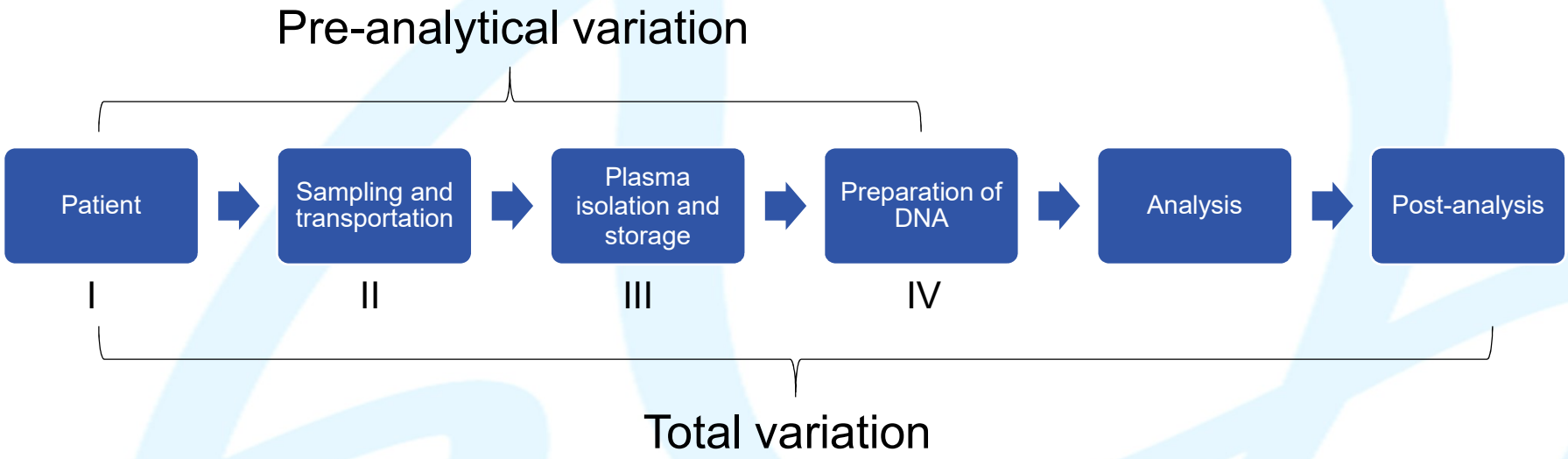

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DNA extraction

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- Manual or automated
- Spin-column or magnetic beads
- cfDNA extraction kit

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Manual vs. automated extraction

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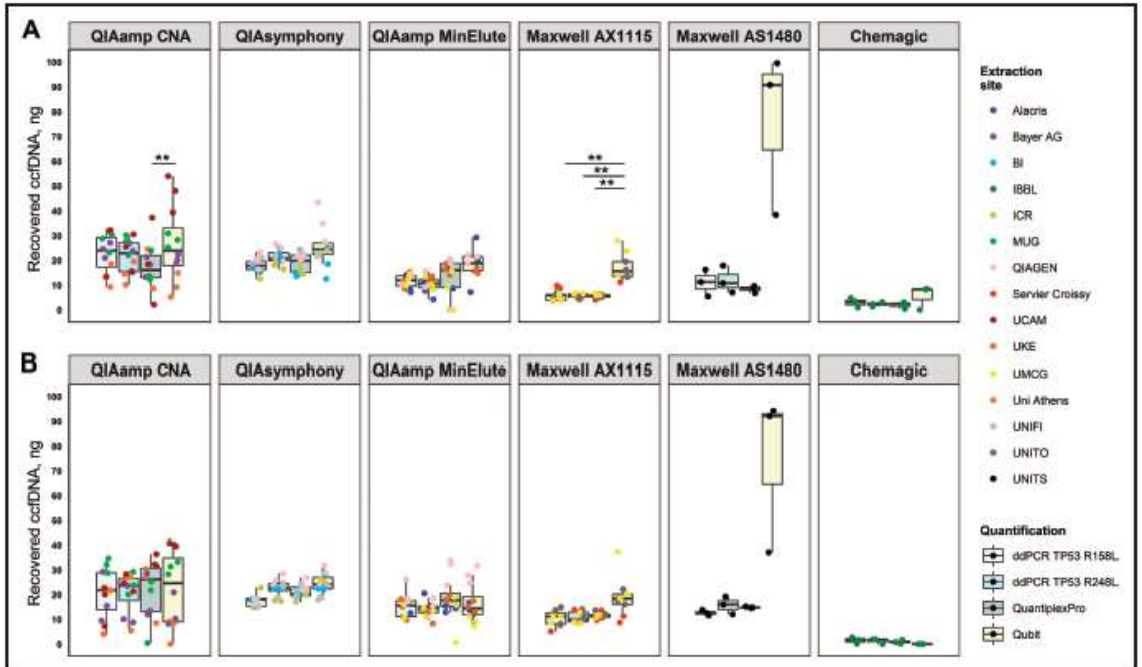

Fig. 2. Multiple comparisons of different ccfDNA extraction methods for spike set I, based on different quantification approaches.
Box plots illustrate the recovery of ccfDNA (spiked mnDNA plus donor-derived ccfDNA) from Streck (A) or PAXgene Blood ccfDNA tubes (B) among 15 extraction sites using 6 commercially available ccfDNA extraction technologies. The yield refers to the recovered nanograms of cfDNA/mnDNA from 4 mL of plasma. Yield was determined by 2 commonly used quantification assays: the qPCR-based Quantiplex Pro assay (gray) and the Fluorometric Quantitation-based Qubit assay (yellow). Moreover, ddPCR data were utilized for quantification, and all positive droplets (mutant and wild-type) from the 2 *TP53* mutation ddPCR assays (R158L, light blue; R248L, blue) were combined and used to calculate the absolute yield of ccfDNA fragments. The horizontal line in each box represents the median. Two-way ANOVA multiple comparison test, $^{**}P < 0.01$.

Clinical Chemistry 66:1
000–000 (2020)

Cancer Diagnostics

Multicenter Evaluation of Circulating Cell-Free DNA Extraction and Downstream Analyses for the Development of Standardized (Pre)analytical Work Flows

Rita Lampignano,[1†] Martin H.D. Neumann,[1†] Sabrina Weber,[2,3] Vera Kloten,[1] Andrei Herdean,[4] Thorsten Voss,[5] Daniel Groelz,[5] Anna Babayan,[6] Marco Tibbesma,[7] Martin Schlumpberger,[8] Francesca Chemi,[9] Dominic G. Rothwell,[9] Harriet Wikman,[6] Jean-Pierre Galizzi,[10] Inger Riise Bergheim,[11] Hege Russnes,[11] Benedetta Mussolin,[12] Serena Bonin,[13] Christine Voigt,[14] Hanny Musa,[15] Pamela Pinzani,[16] Evi Lianidou,[17] Ged Brady,[9] Michael R. Speicher,[2] Klaus Pantel,[6] Fay Betsou,[18] Ed Schuuring,[7] Mikael Kubista,[4] Wim Ammerlaan,[18] Markus Sprenger-Haussels,[8] Thomas Schlange,[1‡] and Ellen Heitzer[2,3*‡] for the Innovative Medicines Initiative CANCER-ID Consortium

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Manual vs. automated extraction

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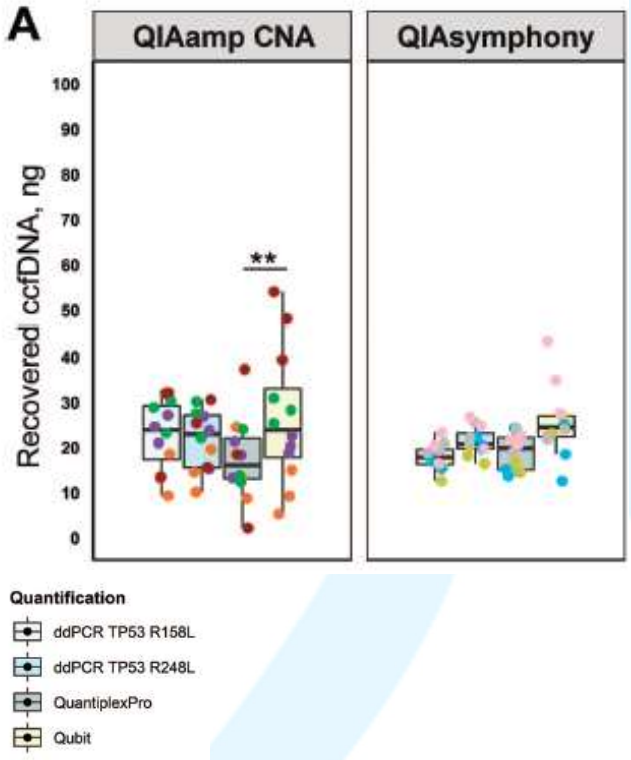

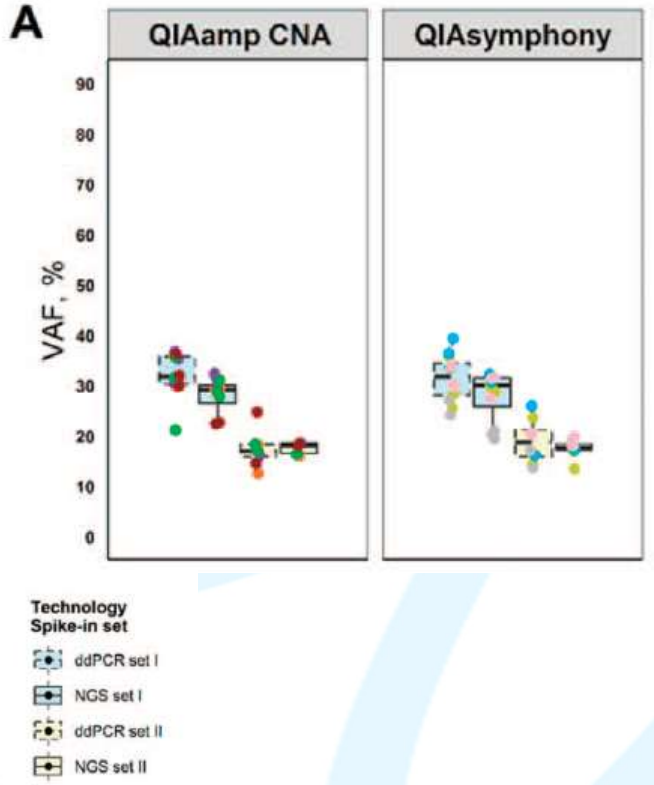

Lampignano R et al. Clin Chem 66:1 (2020)

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Magnetic beads or spin column

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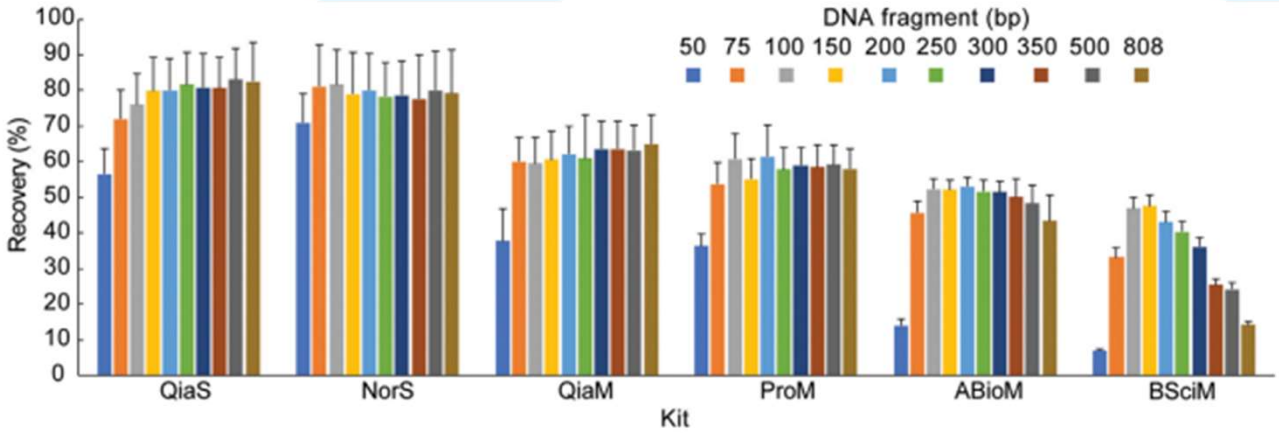

Table 1 Comparison of cfDNA purification kits used in this study.

cfDNA purification kit	Code for this study	Manufacturer	Kit type	Processing time (min)	Sample volume (ml)	Sample type	Price/2 ml sample in manual mode (AUD)	Automation option
QIAamp circulating nucleic acid kit[a]	QiaS	Qiagen	Spin column	90	1–5	Plasma/serum/urine	36.78	QIAcube
Plasma/serum cell-free circulating DNA midi kit	NorS	Norgen Biotek	Spin column	80	1–4	Plasma/serum	33	Manual
QIAamp minelute ccfDNA mini kit	QiaM	Qiagen	Magnetic beads	70	1–2	Plasma/serum	18.86	QIAcube
Maxwell RSC ccfDNA plasma kit[b]	ProM	Promega	Magnetic beads	70	1	Plasma	31.26	Maxwell RSC
MagMax cell-free DNA isolation kit	ABioM	Applied Biosystems	Magnetic beads	70	0.1–10	Plasma/serum/urine	15.92	Kingfisher
NextPrep-Mag cfDNA isolation kit	BSciM	Bioo Scientific	Magnetic beads	60	1–3	Plasma	18.8	Chemagic 360

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Comparison of kits

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- Yield
- Reproducibility
- Fragment lengths
- % tumorspecific DNA
- Double-/singlestranded DNA
- Presence of inhibitors / proteinase K
- Time of procedure, price / ml plasma, automation…

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Quality control of samples

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- Yield (total DNA, exogenous purification control)
- Contamination with DNA from blood cells
- Fragment length (short vs. long fragments)
- Single- vs. doublestranded DNA

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Exogenous extraction control

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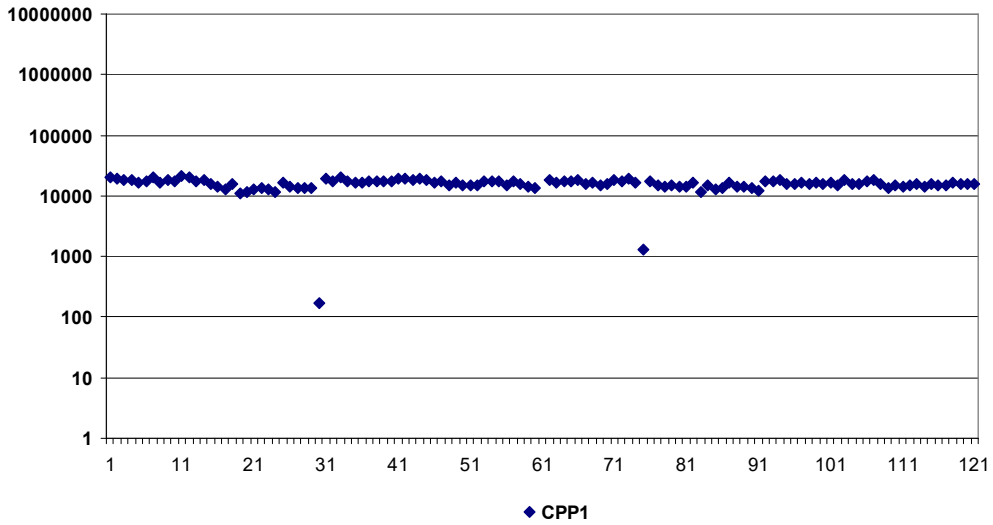

CPP1 alleles in 120 plasma samples

CPP1: exogenous DNA fragment (191 bp) added to plasma samples prior to DNA extraction

Clinica Chimica Acta 446 (2015) 141–146

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Controls to validate plasma samples for cell free DNA quantification

Niels Pallisgaard [a,*], Karen-Lise Garm Spindler [b,c], Rikke Fredslund Andersen [a], Ivan Brandslund [a], Anders Jakobsen [b]

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Fragment length

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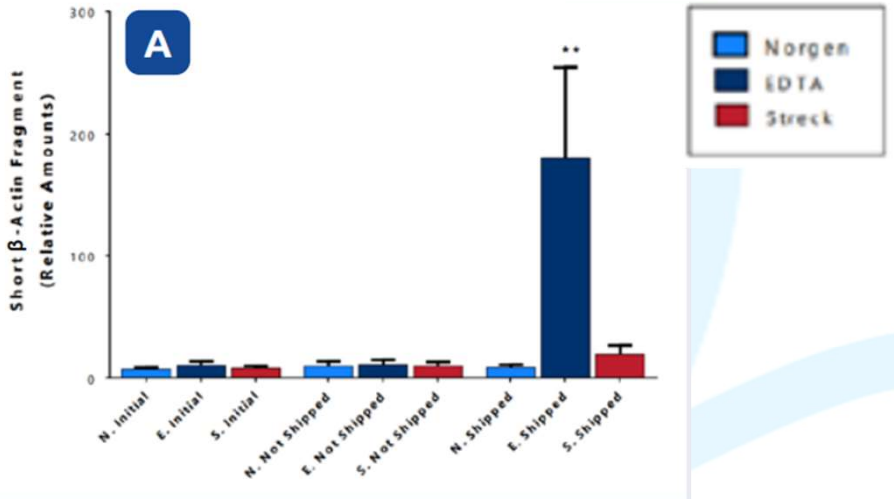
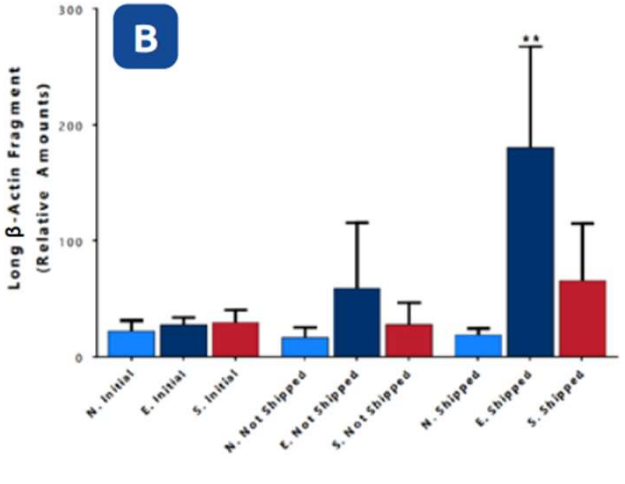
cfDNA is primarily ~160 bp.
Elevated amounts of fragments >200 bp indicates contamination with DNA from blood cells.



136 bp assay vs.
420 bp assay

After 72h, +/- shipment



norgenbiotek.com

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Single- vs. doublestranded DNA

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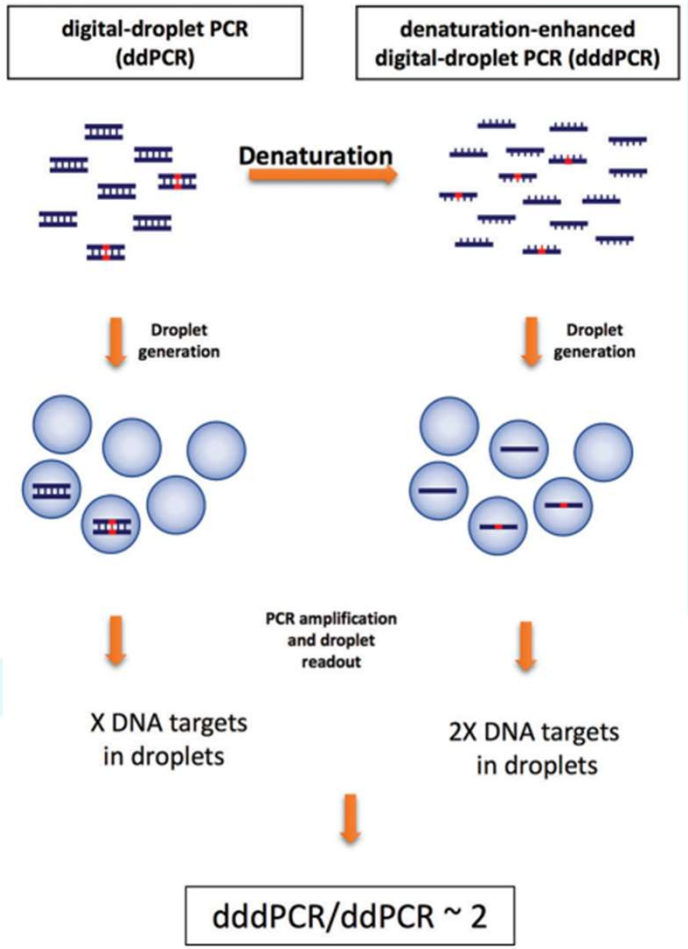
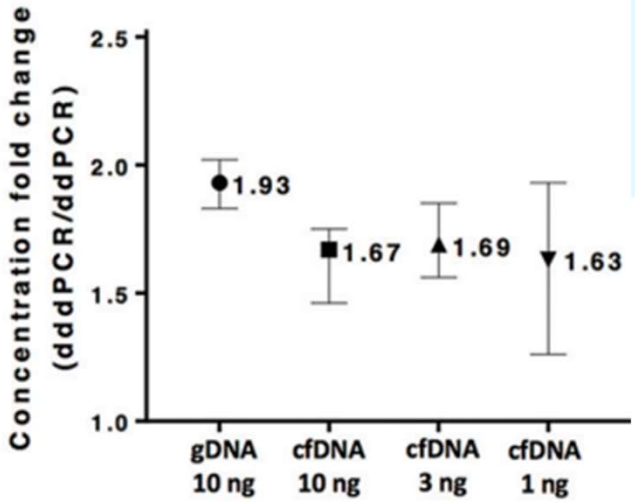


Fitarelli-Kiehl M et al. Clin Chem 64: 12, 1762-1771 (2018)



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Quality control of analyses

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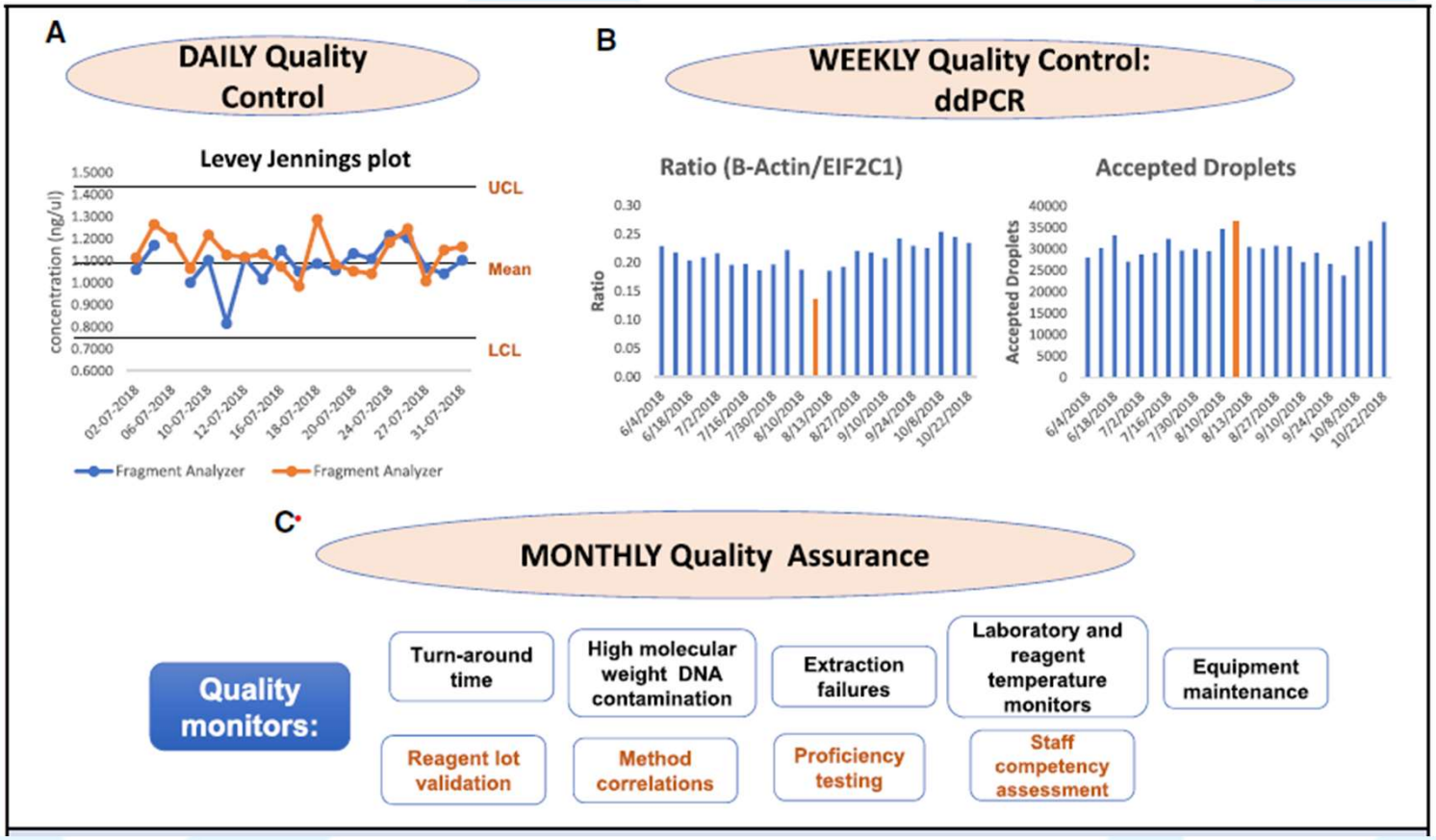

Developing Quality Programs for Cell-Free DNA (cfDNA) Extraction from Peripheral Blood

Aliaksandra Samoila,[a] Jose Sosa,[a] Jessica Padilla,[a] Michael Wutkowski,[a] Katelynd Vanness,[b] Agnes Viale,[b] Michael Berger,[c] Brian Houck-Loomis,[b] Melissa Pessin,[a] and Ellinor I. Peerschke[a,*]

788 JALM | 788–797 | 05:04 | July 2020

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Questions?

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Rikke Fredslund Andersen
Biochemistry and Immunology
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rikke.fredslund.andersen@rsyd.dk

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