Technical Note

Semi-automated RNA Isolation from Tempus Blood RNA Tubes Using the Magcore Plus II Instrument

(total RNA isolation / semi-automated isolation / Tempus Blood RNA / MagCore triXact RNA Kit / next-generation sequencing (NGS))

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Abstract. High-throughput, precise and cost-effective isolation of high-quality RNA is essential for the growing number of RNA-based next-generation sequencing (NGS) analyses. Manual RNA isolation provides sufficient quality but requires significant hands-on time and carries an increased risk of contamination and sample misidentification. Here we describe a semi-automated protocol for the isolation of high-quality total RNA from 3 ml of peripheral blood collected in Tempus Blood RNA Tubes. The isolation can be performed either from the total volume of 9 ml of Tempus blood lysate or from smaller volumes (6 and 3 ml, respectively) using the MagCore triXact RNA Kit on the MagCore Plus II automated nucleic acid extractor, which allows RNA isolation in single tubes. The original isolation protocol (#631) for whole blood RNA isolation was customized by the manufacturer (#631T) by omitting the cell lysis step. After optimizing the process, we compared the yield and quality of 760 RNA samples isolated manually or by semi-automated methods. We conclude that RNA isolation using the semi-automated MagCore protocol yields 5–10 µg of total RNA from 6 ml of lysate (2 ml of peripheral blood), which is almost comparable in quantity and quality to manual isolation. In addition, we show that the remaining 3 ml of lysate is sufficient for backup re-isolation. Our semi-automated RNA protocol reduces hands-on time without increasing costs and yields bulky total RNA of a quality suitable for subsequent RNA NGS applications.

Introduction

RNA isolation is a critical step for many applications, including the RNA-based next-generation sequencing (NGS), which is increasingly used in molecular genetics and pathology in both clinical and research settings (Byron et al., 2016; Karam et al., 2020; Tsimberidou et al., 2022; Horton et al., 2024; Kleiblová et al., 2024). In contrast to DNA, RNA molecules are highly sensitive to degradation, which interferes with downstream applications (Vomelová et al., 2009; Shen et al., 2018). The most common RNA isolation methods require fresh whole blood. To preserve RNA integrity, various protective and storage media have been introduced, including the widely used PAXgene Blood RNA Tubes (Qiagen, Hilden, Germany) and Tempus Blood RNA Tubes (Thermo Fisher Scientific, Waltham, MA; Table 1) (Weber et al., 2010; Franken et al., 2016; Lamot et al., 2019). Their use unifies the pre-analytical phase, allows

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Abbreviations: NGS – next-generation sequencing, OD – optical density, PBS – phosphate-buffered saline, RIN – RNA integrity number, RT – room temperature.

Table 1. Current options for total RNA isolation from stabilized peripheral blood. The total volume of the blood lysate for the RNA isolation is 9.4 ml and 9 ml in PAXgene and Tempus Blood RNA Tubes, respectively.

Extraction kit	PAXgene® Blood RNA Tubes	Tempus® Blood RNA Tubes	EXTRACTION	PRINCIPLE	INSTRUMENT	Number of isolated samples	RNA yield [µg]	RNA purity ($OD_{260/280}$)	RIN (RNA integrity number)
MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit[1]	Y	Y	M	MB	–	1–96 (tubes / 96-well plate)	3–25	1.8–2.2	≥ 7
MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit[1]	Y	Y	A	MB	KingFisher™ Flex[1]	12–96 (strips)	3–25	1.8–2.2	≥ 7
MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit[1]	Y	Y	A	MB	KingFisher™ Duo Prime[1]	96 (96-well plate)	3–25	1.8–2.2	≥7
Preserved Blood RNA Purification Kit II/I[2]	Y	Y	M	SSC	–	(tubes)	5–25	NA	NA
PAXgene Blood RNA Kit (IVD)[3]	Y	N	M	SSC	–	1–12 (tubes)	≥ 3	1.8–2.2	NA
PAXgene Blood RNA Kit (IVD)[3]	Y	N	A	MB	QIAcube®[3]	24–72 (strips)	≥ 3	1.8–2.2	NA
PAXgene 96 Blood RNA Kit (RUO)[3]	Y	N	M	SSC	–	96 (96-well plate)	4–20	1.8–2.2	NA
QIAsymphony PAXgene Blood RNA Kit (RUO)[3]	Y	N	A	MB	QIAsymphony®[3]	96 (96-well plate)	8–16	1.8–2.2	7–9
Tempus™ 12-Port RNA Isolation Kit[1]	N	Y	M	SVSC	ABI PRISM® 6100 Nucleic Acid PrepStation[1]	12 (tubes)	6–25	> 1.9	NA
Tempus™ Spin RNA Isolation Kit[1]	N	Y	M	SSC	–	1–96 (tubes)	6–25	> 1.9	≥ 7
Maxwell® 16 LEV simplyRNA Blood Kit[4]	N	Y	A	MB	Maxwell® 16 Instrument[4]	1–16 (tubes)	NA	NA	NA

1 – Thermo Fisher Scientific, Waltham, MA; 2 – Norgen Biotek, Thorold, Canada; 3 – Qiagen, Hilden, Germany; 4 – Promega, Madison, WI; Y – yes / compatible, N – no / not compatible, A – automated, M – manual, MB – magnetic beads, SSC – silica spin column, SVSC – silica vacuum and spin column, NA – not available.

long-term sample storage at room temperature, improves RNA blood sample handling and eliminates the need for dry ice shipping (Matheson et al., 2008; Weber et al., 2010). Several isolation methods can be used to obtain high-quality RNA from the protected blood samples (Hantzsch et al., 2014; Lamot et al., 2019; Richards et al., 2019). Manual RNA extraction involves many steps with multiple tube changes, which are time-consuming, labour-intensive, and increase the risk of sample contamination or misidentification. On the other hand, RNA isolation using automated or semi-automated extractors allows standardization of sample handling and reduces hands-on time and the risk of sample contamination or misidentification. Several platforms for semi-automated RNA isolation from peripheral blood are currently available, varying in RNA protective solution compatibility, capacity of RNA samples processed per run, input volume of blood sample for the RNA isolation, elution volumes, output RNA isolation format (single tubes vs 96-well plate), RNA yields, and RNA purity (estimated as an optical density ratio of absorbance 260 nm/280 nm; $OD_{260/280}$) (Table 1).

There is currently a limited option available combining the input of popular Tempus blood lysate with RNA elution to 1.5 ml tubes using (semi)automated RNA isolation. Vordenbäumen et al. (2014) previously described the isolation of total RNA from 3 ml of Tempus blood lysate using the MagCore Total RNA Whole Blood Kit (RBC Biosciences, New Taipei City, Taiwan) in a MagCore automated nucleic acid extractor; however, this isolation kit has been discontinued by the manufacturer.

To reintroduce this option, we optimized semi-automated total RNA isolation from the Tempus blood lysate in a widely available MagCore Plus II automated nucleic acid extractor using the MagCore triXact RNA Kit (#631), dedicated by the manufacturer (RBC Biosciences) for RNA isolation from fresh blood but not from the Tempus blood lysate.

Material and Methods

Samples

All peripheral venous blood samples were collected in Tempus RNA Blood Tubes (Thermo Fisher Scientific) and processed as recommended by the manufacturer with vigorous shaking for ≥ 10 seconds before short-

term storage at 4 °C until the isolation (up to 7 days). To optimize the workflow, we used 16 blood test samples from eight healthy volunteers (two samples from each) to assess the quality and quantity parameters (yield, concentration, purity and RNA integrity number – RIN) of the isolated RNA. To demonstrate the feasibility of optimized RNA isolation in routine diagnostic laboratory settings, we compared the workload, quality and quantity of total RNA isolated from 760 blood samples obtained from cancer patients referred for germline genetic testing. RNA from the first half of the samples (380) was isolated manually and the remainder (380) using the optimized semi-automated protocol. Subsequent RNA NGS germline genetic analysis (requiring 200–500 ng of total RNA for NGS library preparation) was performed as described previously (Kleiblová et al., 2024). All individuals gave their informed consent for the analysis approved by the Ethics Committee of the General University Hospital in Prague (No. 22/22 Grant AZV VES 2023 VFN).

Semi-automated total RNA isolation protocol

The first eight test samples were used to assess the yield and quality of total RNA from 9 ml of Tempus blood lysate. The lysate contained 3 ml of peripheral venous blood collected into 6 ml of protective solution in the Tempus Blood RNA Tube. The remaining eight samples were used to test the possibility of re-isolation of backup RNA. To this end, 9 ml of Tempus blood lysate was divided into 6- and 3-ml aliquots.

For the semi-automated RNA isolation using the MagCore triXact RNA Kit on the MagCore Plus II automated nucleic acid extractor, we have optimized the following protocol (Fig. 1):

1. Incubate samples at room temperature (RT) for 2 hours using a rotary mixer at 10 rpm.
2. Transfer 9 ml of Tempus blood lysate into a 15 ml conical tube containing 3 ml of 1 × PBS, vortex vigorously for 30 seconds at RT.*
3. Centrifuge at 4 °C at 4,000 × *g* for 50 minutes, discard supernatant, leave the conical tube inverted on absorbent paper for 1 to 2 minutes.
4. Add 430 µl of cooled 1× RB buffer (supplemented with 1 % mercaptoethanol freshly added before use) to the pellet in the 15 ml conical tube, incubate at 4 °C for 10 min, gently vortex, spin.
5. Transfer the entire volume (~430 µl) to the 1.5 ml sample tube and immediately insert into the MagCore Plus II automated nucleic acid extractor.
6. Run the RNA isolation using cartridge #631 and modified protocol #631T**, elution volume 60 µl, collect into the 1.5 ml elution tube.
7. Measure the quality and concentration of the RNA, store at –80 °C.

Notes:

*Alternatively, transfer 6 ml or 3 ml of Tempus blood lysate into a 15 ml conical tube containing 6 ml or 3 ml of 1× PBS, respectively, vortex vigorously for 30 seconds at RT.

**The modification of protocol #631 for isolation of RNA from unpreserved whole blood samples has been adapted to the processing of Tempus blood lysate by omitting the cell lysis step. The protocol was modified by the manufacturer (RBC Bioscience) and uploaded to the MagCore Plus II automated nucleic acid extractor as protocol #631T. The modified protocol is freely available from RBC Bioscience upon request.

Semi-automated protocol validation

To demonstrate the performance of the optimized semi-automated procedure in routine laboratory settings (using 6 ml of Tempus blood lysate), we compared the yield and quality of RNA obtained from Tempus blood lysate from 380 samples isolated by the semi-automated procedure using the MagCore instrument and 380 samples isolated manually from the entire volume of 9 ml of Tempus blood lysate using the Tempus Spin Kit according to the manufacturer (Thermo Fisher Scientific) (Fig. 1).

Assessment of RNA quality and concentration

Total RNA concentration and purity were measured using NanoDrop One (Thermo Fisher Scientific). RNA integrity was determined using an Agilent RNA 6000 Nano Kit in an Agilent 2100 Bioanalyzer (Agilent Technologies).

Data analysis

The differences in quality and quantity parameters of isolated RNA between semi-automated and manual RNA isolations were analysed using Student's paired *t*-test in R v4.2.2 software.

Results

First, we compared the RNA isolation from eight 9 ml, 6 ml, and 3 ml Tempus blood lysate samples obtained from healthy volunteers. The RNA was isolated using the MagCore triXact RNA Kit (#631) and customized program #631T (described in methods; Fig. 1) on the MagCore Plus II automated nucleic acid extractor. The yield, purity and quality of RNA were comparable between 9 ml and 6 ml isolations (Table 2). As expected, both yield and concentration of RNA obtained from 3 ml were lower with slightly decreased quality (Table 2). Semi-automated isolation from 6 ml of Tempus blood lysate provided sufficient amounts of high-quality total RNA, and the remaining 3 ml of the preserved blood sample could be used as a backup for confirmatory purposes or in case the isolation procedure failed. Therefore, we decided to routinely perform RNA isolation from 6 ml of Tempus blood lysate.

To compare semi-automated and manual RNA isolation in routine settings, we processed 760 blood samples from cancer patients, of which 380 were isolated using the semi-automated protocol from 6 ml of Tempus blood

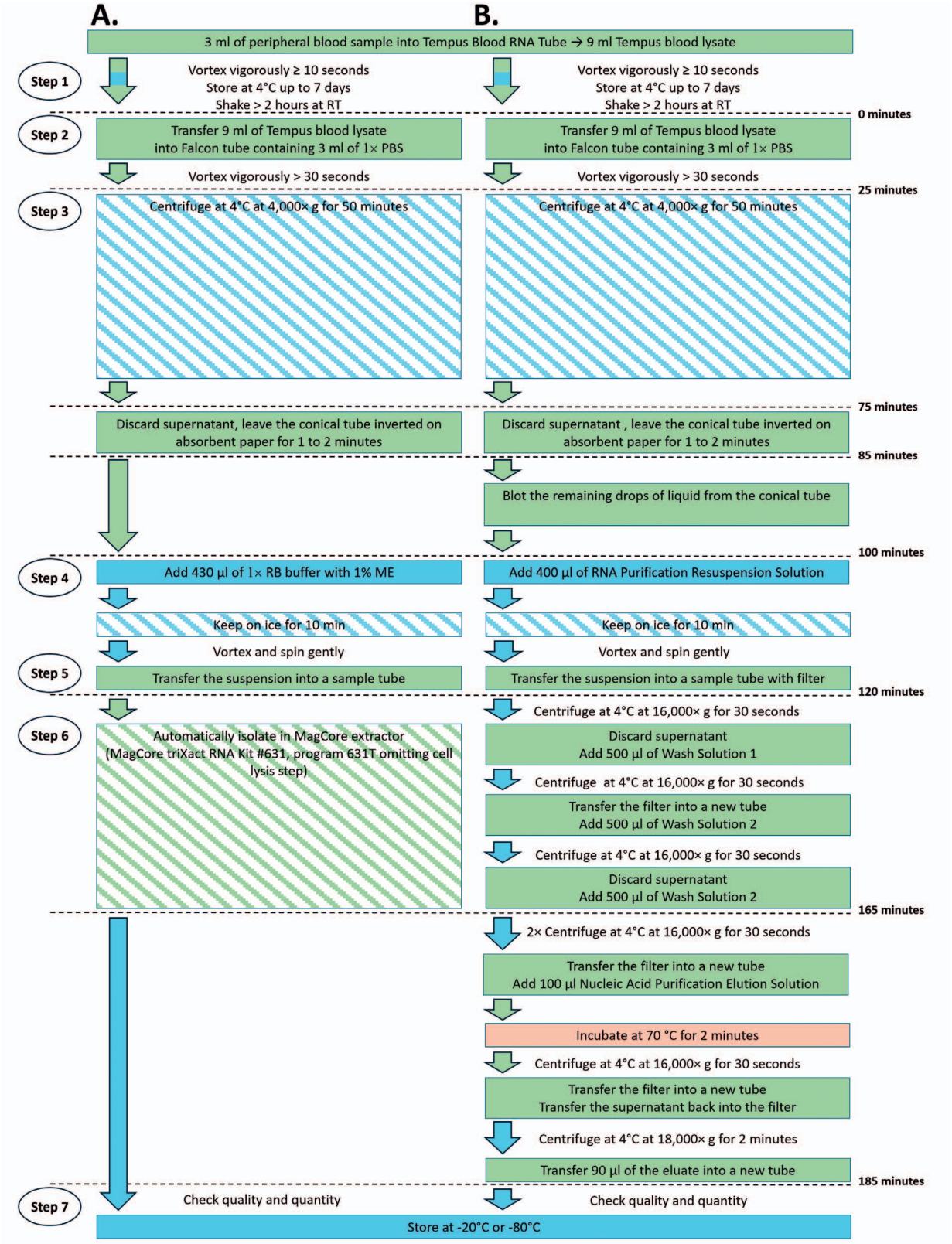

Fig. 1. Workflow of the semi-automated RNA isolation from 9 ml of Tempus blood lysate using **(A)** semi-automated isolation using the MagCore triXact RNA Kit (#631) with customized program #631T without DNase treatment on the MagCore Plus II automated nucleic acid extractor; **(B)** manual isolation using the Tempus Spin RNA Isolation Kit (according to the manufacturer's instructions with minor modifications to increase the yields – the centrifugation time was extended to 50 minutes and the speed was increased to 4,000 × *g*). Steps highlighted in green are performed at room temperature, steps highlighted in blue are performed on ice or in cooled instruments. Steps highlighted in hatched colours are automated without the operator's supervision. The time estimates show the typical workload for processing 16 samples (ME, mercaptoethanol; RT, room temperature).

Table 2. Comparison of mean qualitative and quantitative parameters of the semi-automatically isolated RNA (eluted to 60 µl of elution solution) obtained by various volumes of Tempus blood lysate and PBS

Tempus blood lysate [ml]	1×PBS [ml]	Samples (N)	RNA yield [µg]	RNA concentration [ng/µl]	RNA purity ($OD_{260/280}$)	RIN
9	3	8	7.6 ± 2.8	126.5 ± 46.2	2.10 ± 0.01	7.4 ± 0.3
6	6	8	9.3 ± 2.2	154.7 ± 37.0	2.10 ± 0.03	7.5 ± 0.4
3	3	8	4.0 ± 0.9	67.4 ± 14.4	2.20 ± 0.06	6.9 ± 0.8

RIN – RNA integrity number

lysate and 380 others were isolated using the manual Tempus Spin Kit from 9 ml of Tempus blood lysate (according to the manufacturer's instructions; Fig. 1). The mean yields were 7.8 µg and 18.2 µg (mean concentration 130.6 and 202.2 ng/µl; $P < 0.001$) for semi-automated and manual isolation, respectively, with a mean purity ratio assessed by $OD_{260/280}$ of 2.1 for both procedures. The quality of isolated RNA (RIN) was assessed for a randomly selected subset of 33 samples isolated using the semi-automated protocol and 22 samples isolated manually. The RINs obtained for the procedures were higher for manually isolated RNA (7.5 vs 7.0; $P = 0.05$; Fig. 2). Nevertheless, all samples isolated by either semi-automated or manual isolation were successfully used for preparation of sequencing libraries and seamlessly analysed by targeted RNA NGS germline genetic analysis (data not shown), as we demonstrated previously (Kleiblová et al., 2024).

Regarding the workload, the manual RNA extraction of a full batch of 16 samples lasted 185 minutes, with 125 minutes of operator hands-on time. Semi-automated RNA extraction of 16 samples required 150 minutes, with 45 minutes of operator hands-on time.

Semi-automated isolation provided a lower RNA yield (which could be partly explained by the lower starting lysate volume: 9 ml vs 6 ml), comparable RNA quality and significantly shorter hands-on time compared to the manual method.

Discussion

The vast majority of automated approaches to RNA isolation from preserved samples (Tempus or PAXgene) allow isolation in strips or 96-well plates rather than individual tubes, which can be a limiting factor for long-term storage and repeated handling of RNA samples.

Combining the input of Tempus blood lysate with the collection of RNA in 1.5 ml single tubes is currently almost impossible. Elution into individual tubes avoids unnecessary thawing of RNA samples not currently used for the analysis.

Therefore, we adapted the RNA isolation protocol (Fig. 1) with the input of 9 ml Tempus blood lysate (equivalent to 3 ml of whole blood) using the MagCore triXact RNA Kit (#631) and the manufacturer's customized program #631T (#631 without cell lysis) using the MagCore Plus II automated nucleic acid extractor. Our optimized semi-automated RNA extraction on the MagCore Plus II thus eliminates the need for a dedicated RNA extractor. The semi-automated protocol yielded RNA of similar integrity (RIN 7.4) and RNA concentration (Table 2) as previously reported by Vordenbäumen et al. (2014) using the same device but with the kit no longer available from the manufacturer (Vordenbäumen et al., 2014). Moreover, the isolation of 16 samples in parallel using the semi-automated protocol reduces hands-on time when compared to manual isolation.

All 380 RNA samples isolated by the semi-automated procedure in routine laboratory settings from 6 ml of Tempus blood lysate (equivalent to 2 ml of whole blood) were successfully used for subsequent targeted RNA-NGS germline genetic analysis, as we described previously (Kleiblová et al., 2024). In addition, the testing re-isolation from the remaining 3 ml aliquot of Tempus blood lysate demonstrated that the 3 ml aliquot could be used as a backup, providing total RNA with still sufficient quality and concentration for most downstream applications.

The automated part of the RNA extraction was performed by the MagCore Plus II automated nucleic acid extractor, which is used by many mid-size diagnostic/research laboratories for DNA extraction.

Conclusion

We have shown that the RNA isolation protocol that combines the advantages of blood collection with RNA preservation in Tempus Blood RNA Tubes and semi-automated RNA isolation using the MagCore triXact RNA Kit (#631) on the MagCore Plus II automated nucleic acid extractor (RBC Biosciences) using the manufacturer's customized program #631T provides collection of high-quality RNA in 1.5 ml collection tubes with reduced hands-on time.

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Data availability statement

The data presented in this study are included in this publication or are available from the corresponding author upon reasonable request.

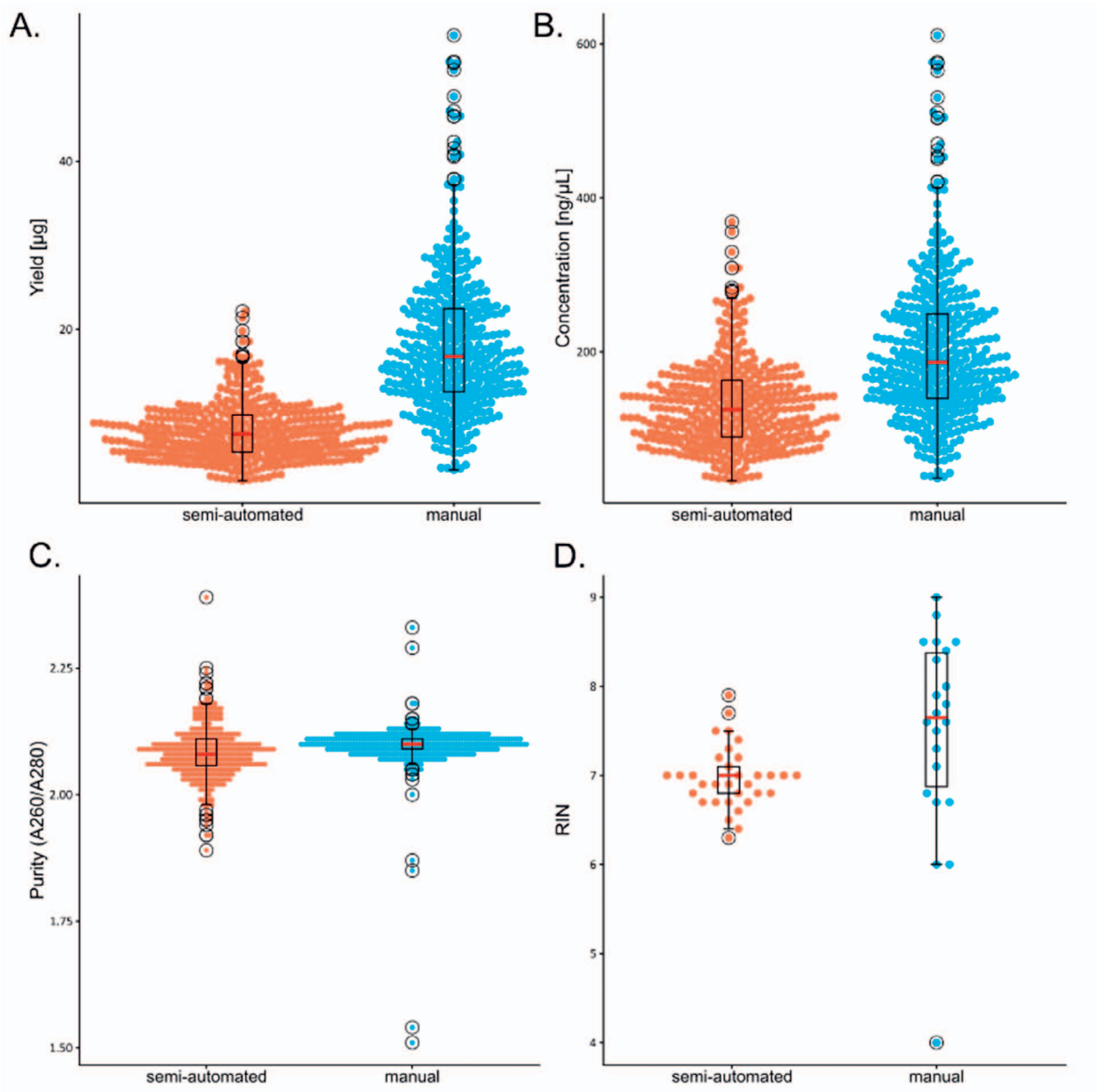

Fig. 2. The **(A)** yield, **(B)** concentration, **(C)** $OD_{260/280}$ ratio and **(D)** RIN* of the isolated RNA from 760 cancer patients – 380 were isolated using the semi-automated protocol on the MagCore Plus II instrument (from 6 ml of Tempus blood lysate; elution volume 60 µl; orange) and 380 were isolated manually by the Tempus Spin Kit (from 9 ml of Tempus blood lysate; elution volume 90 µl; blue). *RIN was assessed only for 33 and 22 samples isolated by the semi-automated protocol and manually, respectively.

Conflict of interest

We declare that this work was performed in cooperation with KRD, on behalf of RBC Biosciences. The authors declare no other potential conflict of interest. The authors declare that the results summarized in this manuscript have not been published previously and have not been submitted for consideration to any other journal.

Authors' contributions

MČ: Methodology; Investigation; Formal analysis and data curation; Writing – Original draft. BŠ: Methodology; Investigation; Formal analysis and data curation; Writing – Original draft. PP: Investigation. TP: Investigation. MJ: Writing – Editing. JS: Writing – Editing. KH: Conceptualization and methodology; Investigation; Formal analysis and data curation; Writing – Original Draft. PK: Conceptualization and methodology; Investigation; Formal analysis and data curation; Writing – Editing; Resources; Funding acquisition.

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