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Letter to the editor

Assessment of magnetic bead-based automated whole blood RNA-isolation from a validated RNA stabilization reagent (Tempus Blood RNA)

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We read with great interest the article of Matheson et al. (2008) who compared two RNA stabilizing reagents (Tempus Blood RNA and PAXgene) concerning RNA yield, RNA quality, and the cost of RNA isolation. In their study, the isolation of total RNA out of stabilized blood was carried out manually according to the manufacturer's protocols. The use of Tempus Blood RNA resulted in a higher yield of total RNA, better measures of quality, and lower costs. Matheson et al. emphasized the importance of effective and reasonably priced RNA preservation in the research on rare diseases, such as the majority of autoimmune rheumatic diseases, which often require a collaborative effort of large and small centers. Important obstacles for the routine storage of such RNA samples in biorepositories consist in the need for reliable RNA preparation of high quality despite transportation, the time-consuming isolation of RNA, and high costs, amongst other reasons. In addition to the study by Matheson et al., we were thus interested to compare a manual and an automated approach to the isolation of total RNA from whole blood stabilized with Tempus Blood RNA to assess further potential for a time- and cost-efficient approach to obtain high quality RNA samples.

Blood from eight healthy individuals (no chronic or current acute disease, no over the counter or prescribed medication, full informed consent, 4 males (33.3 ± 9 years), 4 females (33.8 ± 15 years)) was collected into 2 Tempus Blood RNA tubes (3 ml each) and stored for 24 h at room temperature to simulate transportation. The content of each tube was then used for ternary isolation of RNA either by using the Tempus Spin RNA Isolation Kit according to manufacturer's instructions (Applied Biosystems, Warrington, UK; manual) or by a RNA isolation kit which has recently been optimized for automated isolation of RNA from Tempus Blood RNA on the MagCore Nucleic Acid Extractor (MagCore Total RNA Whole Blood Kit, RBC Bioscience, New Taipei City, Taiwan; automated). Briefly, 3 ml solution per Tempus Blood RNA tube, corresponding to 1 ml blood and 2 ml stabilization reagent, was washed in PBS and centrifuged, and pellets treated with DNase, lysed in mercaptoethanol, and subsequently inserted into the MagCore instrument for further automatic RNA isolation. Hands-on time of the technician was recorded for both procedures. Afterwards, the total RNA content and absorbance at 230, 260 and 280 nm were measured by NanoDrop 1000 (Thermo Scientific, Waltham, USA) in order to estimate the purity of extracted RNA by calculating 260/280 and 260/230 ratios. Moreover, the RNA Integrity Number (RIN) was determined on an Agilent 2100 Bioanalyzer using Small RNA Chips (Agilent Technologies, Santa Clara, USA) as a general measure of RNA quality.

Automated RNA isolation (triplicate RNA isolation of 4 probands) was accomplished after 79.5 ± 4 min compared to 130 ± 4 min hands-on time when employing manual isolation ($p = 0.0059$, t-test). RNA yield was up to 3-fold lower with automated compared to manual isolation: 47.8 ± 7.8 ng/ml and 21.0 ± 4.3 ng/ml, respectively (mean ± SD, $p = 0.0078$, Wilcoxon matched pair signed rank test). The fluctuation range (FR) of the RNA yield (range of RNA yield divided by the mean of 3 measurements) was comparable with 12.2% (interquartile range (IR) 6.9–18.5%) for the manual and 14.7% (IR 6.5–22.8%) for the automated method. RNA quality was assessed by calculation of spectrophotometric 260 nm/280 nm and 260 nm/230 nm absorbance ratios as well as the determination of the RIN. Table 1 shows that RNA quality was slightly, but significantly lower after the automated compared to manual RNA isolation in all parameters assessed. Furthermore, FR for the RNA quality parameters was calculated to estimate the reliability of manual and automated isolation: FR for 260/280 absorption was 5.0% (IR 2.2–8.0%) and 9.7% (IR 5.1–13.2%), FR for 260/230 was 14.0% (IR 8.8–16.4%) and 27.7% (IR 7.8–42.5%), FR for RIN was 12.6% (IR 3.0–13.4%) and 7.8% (3.0–9.6%) ($p < 0.05$ according to Wilcoxon matched pair signed rank test for all comparisons).

Hence, automated RNA isolation from Tempus Blood RNA using a bead-based approach resulted in obvious savings of time at a considerable cost of RNA yield. Importantly, lower total RNA yield does not automatically preclude further analyses. This was shown by Prezeau et al. who similarly found satisfactory RNA quality for the detection of residual hematological disease despite a reduced total amount of RNA extracted from Tempus Blood RNA (Prezeau et al., 2006). RIN, the most robust marker for RNA quality assessment in our analysis, was only numerically slightly, nevertheless significantly, lower in the automated analysis. These results strongly imply that any stabilization reagent and isolation procedure have to be validated against alternative techniques for every

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Table 1
Comparison of RNA quantity and quality after manual and automated RNA isolation from Tempus Blood RNA.

	RNA yield [μg/ml]	260/280	260/230	RIN
Manual	47.8 ± 7.8	2.1 ± 0.0	1.9 ± 0.3	8.0 ± 0.6
Automated	21.0 ± 4.3	1.9 ± 0.1	1.3 ± 0.4	7.5 ± 0.6
p-Value	<0.05	<0.05	<0.05	<0.05

Mean ± SD of RNA yield and calculation of spectrophotometric absorbance measurement ratios at 260, 280, and 230 nm, representing purity of extracted RNA (determined by NanoDrop 1000). Mean ± SD of RNA Integrity Number (RIN) measured on Agilent 2100 Bioanalyzer. p-Values reported according to Wilcoxon matched pair signed rank test.

consequently analyzed parameter. This notion is further stressed by the findings of Nikula et al. who demonstrated that the choice of mRNA stabilization reagent differentially influences the detection of expressed genes (Nikula et al., 2013). Furthermore, extrapolations concerning RNA quality based on previous assessments of the stabilization reagent alone, without consideration of the RNA extraction method used or the consequent test applied (e.g. detection of residual disease, gene-array experiments, targeted quantitative PCR of single mRNAs) may result in falsely rejecting or accepting the method at hand (Matheson et al., 2008; Prezeau et al., 2006; Nikula et al., 2013). Finally, shipping or storage conditions in biorepositories may have to be accounted for and should influence the choice of stabilization reagent and isolation procedure (Matheson et al., 2008; Prezeau et al., 2006; Nikula et al., 2013; Weber et al., 2010; Duale et al., 2012). Although limited by small proband number, our findings further support the notion that sufficient RNA quality control is necessary for valid analyses. In conclusion, automated isolation of RNA from Tempus Blood RNA by a bead-based approach results in lower RNA quantity and slightly lower quality. Time-savings may justify the use of this method if validity of the results is demonstrated for the analyzed target mRNA in comparison to the manual method.

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