Comparing RNA extraction methods to face the variations in RNA quality using two human biological matrices.

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Abstract

Nucleic acids, *RNA* among them, are widely used in biomedicine. Because of its susceptibility to degradation by RNases, the handling and extraction process of *RNA* from cells and tissues require specialized personnel and standardized methods to guarantee high purity and integrity. Due to the diversity of techniques found in the market, a comparative study between different *RNA* extraction methods is useful to facilitate the best choice for the researcher. In this study, we have compared seven different *RNA* extraction methods (automated, semi-automated, and manual) from two biological matrices: human Jurkat T cells and peripheral blood mononuclear cells (PBMC). Results showed marked differences in the *RNA* quality, and functionality according to the method employed for *RNA* extraction and the matrix used. These data contribute to facilitate researchers in decision-making practices and emphasize the relevance of the selection of the *RNA* extraction method in each experimental procedure to guarantee both quality standards and its reproducibility.

Declarations

Author Disclosure Statement

The authors have no conflicts of interest to declare.

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Author contributions

JOP and PF planned and performed the experiments. JOP, PF, JMGZ, and ALS analyzed data. LL, MJPM, BO, BM, MVB, and PJRB provided feedback on all steps of the search and data extraction, LL, MJPM, BO, GET and PJSC oversaw the project and provided advice. GET reviewed the manuscript and assisted with data analysis. JOP and PF wrote the manuscript.

Ethical approval and consent to participate

All applicable international, national, and/or institutional guidelines were followed. All the enrolled volunteers gave their written informed consent obtained from the BBSSPA and approved by the Ethics Committee of Clinical Research (CEIC) from IBIMA. The study was carried out in accordance with The Code of Ethics of the WMA (Declaration of Helsinki).

Consent for publication

All authors have directly participated in the planning, execution, or analysis of this study and approved the contents of the manuscript and its submission.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Introduction

Nucleic acids are widely used in biomedical research and clinical practice. Specifically, ribonucleic acid (*RNA*) is found in every living cell and implicated in multiple functions, due to the large existing *RNA* variety (Cooper et al. 2009; Zhang 2009; Liu et al. 2017). Thus, m*RNA*, *RNA*i, small *RNA*, or micro*RNA*, among others, have been key to improve understanding of gene expression control for the study, diagnosis, or treatment in many pathologies (Hawkins and Morris 2008; Mihailescu 2015, Bai et al. 2013; Ferro et al. 2016; Martínez et al. 2018). Because *RNA* degradation is critical (Arraiano et al. 2010; Optiz L et al. 2010) it is advisable to use standardized work protocols that guarantee the quality of the material. The use of commercial or automated methods against manual protocols minimizes the exposure to degradation agents and sample handling, reducing the probability to undergo degradation (Thatcher 2015).

Currently, there are several indicators of *RNA* quality. For *RNA* purity, spectrophotometry by evaluating the 260/280 and 260/230 ratios or fluorimetry using the Quant-iT ™ RiBogreen® *RNA* can be used (Fleige and Pfaff 2006; Aranda et al. 2009; Lucena-Aguilar et al. 2016). Fluorometry quantification has several advantages over spectrophotometry, because of its accuracy. However, it has some drawbacks such as giving bad quantification in samples with very low *RNA* concentrations, and its high cost (Deng et al. 2005). *RNA* integrity can be evaluated by agarose gels to detect 28S, 18S, and 5S ribosomal *RNA*, or by the electrophoretic-based generation using Agilent's Bioanalyzer (Agilent Technologies, Inc, Santa Clara, CA, USA), while functionality can be tested by conventional polymerase chain reaction (PCR) or real-time PCR (Umaña et al. 2013). Obtaining *RNA*s with optimal values for all these indicators is essential to generate reliable results.

Ideal *RNA* extraction method should be simple, fast, economical, reproducible, with low variability between samples, and able to maintain *RNA* purity and integrity. Comparative studies from different species (hernández-Guzmán and Gzumán-Barney 2013; Oliveira-Alves et al. 2016; Chauan et al. 2018; Gan et al. 2016), and matrices, including saliva, whole blood, or skin (Ruettger et al. 2010; García-Nogales et al. 2011; Bayatti et al. 2014; Madera-Anaya and Suárez-Causado 2017; Reiman et al. 2019), revealed differences in the yield, quality, and functionality of *RNA* obtained. However, at present there are no studies comparing a substantial number of *RNA* extraction methods and, specially, using the so extensively studied PBMC or human Jurkat T cells.

In this study, we have compared seven *RNA* extraction methods including manual (TRIzol™), semiautomated (QIAGEN™, Bio-Rad, Monarch®, and Canvax™), and fully automated (QIAcube™ and Maxwell®) process. *RNA* samples were evaluated by spectrophotometry and fluorimetry to determine their yield and purity. Subsequently, an assessment of integrity and a functionality study was carried out. Finally, a determination of cost, process time, and other related factors were presented. This study is the first to show a comprehensive comparative study of *RNA* extraction and in PBMC and Jurkat T cells. Therefore, this report also contributes to helping researchers in decision-making protocols and validity of laboratory *RNA* methods, according to their requirements.

Materials And Methods

Cell cultures

Human Jurkat T cells were grown in RPMI-1640 with 10% heat-inactivated fetal calf serum, 10mM HEPES, 2.5 g/l glucose, 1 mM sodium pyruvate, and 25 µg/ml gentamicin (all products from Merck Life Science SLU, Spain) at 37 ºC in a humid atmosphere of 5% CO_2. Confluent cell cultures were centrifuged to harvest cells and washed in Dulbecco phosphate-buffered saline (DPBS) without calcium or magnesium. After centrifugation, the pellet was resuspended in DPBS. Live cells were estimated by trypan blue exclusion. Aliquots of 5×10^6 cells were cryopreserved in RPMI supplemented with 40% FBS and 10% dimethyl sulfoxide (DMSO) and stored at -196 °C until *RNA* extraction.

Subjects

Blood samples were obtained from 30 volunteers, collected in 4 ml EDTA BD Vacutainer™ tubes (Becton Dickinson & Company, New Jersey, USA). After processed, the samples were stored at -196 ºC until use, in the Biobank platform of the IBIMA, which is part of the Biobank of the Andalusian Public Health System (BBSSPA). All the enrolled volunteers gave their written informed consent obtained from the BBSSPA and approved by the Ethics Committee of Clinical Research (CEIC) from IBIMA. The study was carried out in accordance with The Code of Ethics of the WMA (Declaration of Helsinki).

PBMC purification

The procedure was carried out as previously described by Ortega-Pinazo et al. (2019). Briefly, Lymphosep™ (Biowest, Riverside, Missouri, USA) was added to a sterile tube, blood was diluted 1:1 with physiological saline and gently deposited in these tubes. A density gradient was established by centrifugation at 616 ´g for 25 min without braking. PBMC layer was collected and transferred into a new tube. Then, two washing steps with physiological saline were performed, the first at 616 ´g for 10 min to remove the Lymphosep™ surplus and the second at 122 ´g for 10 min to remove platelets. The supernatant was discarded, and the pellet was resuspended in DPBS. Live cells were estimated by the exclusion of trypan blue. Aliquots of 3.5×10^6 cells were cryopreserved in RPMI supplemented with 40% FBS and 10% DMSO and stored at -196°C.

RNA extraction

Cells were thawed and washed twice with DPBS without calcium or magnesium at 122 ´g for 8 min to remove DMSO. Aliquots of 3×10^6 viable cells were prepared in DPBS as indicated in the product manuals. *RNA* was extracted using seven different methods: 2 automated methods (Maxwell® and QIAcube™), 4 semiautomated methods (QIAgen™, Bio-Rad, Monarch®, and Canvax™), and 1 manual method (TRIzol™). After extraction, *RNA* samples were resuspended in 30 µl of sterile ultrapure water and stored at -80 ºC until use. - **Maxwell®:** *RNA* was extracted from samples with the commercial Maxwell® 16 Total *RNA* Purification Kit (Promega, Wisconsin, USA) using the Maxwell® robot following the manufacturer´s instructions.- **QIAcube™:** Previously, DPBS was removed from samples by cold centrifugation at 122 ´g for 8 min. The supernatant was discarded, and the pellet was resuspended in 750 µl of TRIzol™ (Invitrogen, Thermo Fisher Scientific, Massachusetts, USA) and stored at -80 ºC for 48 hours. Then, samples were thawed, and *RNA* was extracted with the commercial miRNeasy mini Kit (QIAgen™, Hilden, Germany) using the QIAcube™ robot, following the manufacturer´s instructions.- **QIAgen™:** First, DPBS was removed and the pellet was processed and stored as previously cited. Then, samples were thawed, and *RNA* was manually extracted with the commercial QIAmp™ RNeasy mini Kit (QIAGEN™), following the manufacturer´s instructions.- **Bio-Rad:** *RNA* was extracted with the commercial Aurum™ Total *RNA* Mini Kit (Bio-Rad, California, USA), following the manufacturer´s instructions. - **Monarch®:** *RNA* was extracted with the commercial Monarch® Total *RNA* Miniprep Kit (New England Biolabs, Massachusetts, USA), following the manufacturer´s instructions.- **Canvax™:** *RNA* was extracted with the commercial HigherPurity™ Total *RNA* Extraction Kit (Canvax™ Biotech S.L., Córdoba, Spain), following the manufacturer´s instructions.- **TRIzol™:** Previously, DPBS was removed from samples by cold centrifugation at 122 ´g for 8 min and the pellet was incubated with 800 µl of TRIzol™ (Invitrogen) at RT for 10 min with agitation. Then, 200 µl of chloroform was added and after manual agitation for 15 s, samples were cold centrifuged at 12000 ´g for 15 min, the transparent phase was collected for *RNA* extraction and transferred to a new sterile tube adding 600 µl of isopropanol. After 20 min incubation at -20 ºC, samples were cold centrifuged at 12000 ´g for 10 min. The pellet was washed once with 1 ml of ethanol 75%. After cold centrifugation at 7500 ´g for 5 min, the pellet was dried to remove the excess ethanol.

RNA quantification, purity, and integrity

Absorbance at 260, 280, and 230 nm of 2 µl of each *RNA* sample was measured in duplicate using the NanoDrop™ 2000 (ThermoFisher Scientific). Ultrapure water was used as blank. The concentration of *RNA* from 260 nm absorbance was calculated according to the Lambert-Beer law. The 260/280 ratio (i.e., *RNA*/protein) was used as a purity indicator. Optimum 260/280 ratio values or pure *RNA* ranged from 1.8 to 2. The 260/230 ratio (*RNA*/contaminants) was used as a secondary measurement of purity, establishing the optimal, in this case, values > 1.8 (Desjardins and Conklin 2010). The concentration of *RNA* was also performed using a fluorometric method. Thus, Qubit *RNA* HS Assay Kit (Life Technologies, Thermo Fisher Scientific,) was used following the manufacturer's instructions. First, two standards were assessed to generate a standard curve. Subsequently, the Qubit™ assay reagent

was added to the samples and processed into the reader Qubit™ 3.0 Fluorimeter independently. The integrity of total *RNA* was determined by electrophoresis. Samples were separated on 3% agarose gels stained with SYBR Safe (Invitrogen) in running buffer (1×Tris/acetic acid/ethylenediaminetetraacetic acid, pH 8.0). Gel images were analyzed using ImageQuant™ LAS 4000 (GE Healthcare, Illinois, USA).

RNA functionality

RNA functionality was evaluated by PCR amplification or real-time PCR assay. To obtain the c*DNA*, an aliquot containing 1 µg of total *RNA* was reverse transcribed using M-MLV reverse transcriptase (Merck Life Science), 5 µM random hexamers, and 2 mM dideoxynucleotides (Roche, Basilea, Switzerland) in a total reaction volume of 20 µl, following the manufacturer´s instructions. Samples were stored at -20ºC. For PCR, a fragment of 312 base pairs of the HIST1H4A gene was amplified following the protocol previously published by Ortega-Pinazo et al. (2019). For the real-time PCR assay, 25 ng of c*DNA* from each sample was amplified for the *PRKG1* and *IMPDH2* genes obtaining a PCR product of 74 and 98 bp, respectively, in an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystem, California, USA) using iTaq™ Universal SYBR Green Supermix (Bio-Rad). Triplicate determinations were performed, and the C_T value was analysed. The primer sequences for real-time PCR were: forward 5'-CCACCGCCTTCGACAT- 3' and reverse 5'-CCTGCTTACTGTGGGCTCTTG- 3', for the PRKG1 gene; and forward 5'-CCATCTCATCCCTGCGTGTCTCCGAGGACT- 3' and reverse 5'-CCTATCCCCTGTGTGCCTTGCCTCCCACGACT- 3', for the IMPDH2 gene. PCR and real-time PCR products were assessed on a 2.5% denaturing agarose gel stained with SYBR Safe (Invitrogen).

Statistical studies

Data were presented as the mean values as means ± SD. Statistical analyses were performed using the R program and SPSS (v20.0). The significance between groups was determined with the Student´s and one way ANOVA test; Only probabilities ≤ 0.05 were considered significant. The Tukey test was used to perform multiples comparison between the variables.

Results And Discussion

Concerning the yield of the extraction process for each of the methods tested in human Jurkat T cells, ANOVA and Tukey analysis on data from the quantification by spectrophotometry using NanoDrop™ system revealed significant differences ($P < 0.001$) for all methods tested. Pair-by-pair comparison showed statistically significant differences for all methods, except for QIAgen™ vs. QIAcube™, and Canvax™ vs Maxwell® methods which do not substantially differ. The highest yields were obtained using TRIzol™, QIAgen™, or QIAcube™ (Table 1). The semiautomated Canvax™ and automated Maxwell® extraction methods provided worse *RNA* yields with respect to the rest of the methods. Similarly, quantification by fluorimetry using Qubit™ showed, comparatively, a significant ($P < 0.001$) higher performance in the *RNA* samples obtained with TRIzol™, QIAgen™, or QIAcube™ methods, and a substantially lower performance with Bio-Rad, Canvax™, and Maxwell®, respectively (Table 1). In all

cases, the yield was within the range proposed for each method by its respective manufacturer. Six extraction methods were tested (QIAgen™, QIAcube™, Bio-Rad, Monarch®, Maxwell®, and TRIzol™) in PBMC. Only QIAcube™ and TRIzol™ methods showed statistically significant pair-by-pair differences and the highest yields (Table 1). Fluorimetry confirmed the results, showing the lower performance when Maxwell® and Bio-Rad were used. All results were within the range proposed for each method by its respective manufacturer. Besides, a remarkable difference was observed depending on the matrix used for extraction. Best yields were obtained for the human Jurkat T cells, reaching up to 80% higher in QIAcube™ and QIAgen™ methods, remaining unchanged for the Maxwell® method.

From these findings, the best yield was obtained for QIAcube™ (automated), QIAgen™ (semi-automated), and TRIzol™ (manual) methods. In line with our results from Jurkat T cells, a previous work from Tavares et al. (2010) using SK-N-MC neuroblastoma cells reported that semi-automated methods had better yield than manual ones. However, for PBMC, a yield up to 1.18 times higher was found in manual extraction methods when were compared with commercial kits (Ruettger et al. 2010, Oliveira-Alves et al. 2016), in agreement with our study. The decrease in the yield of some semi-automated methods, such as Bio-Rad and Canvax™, may be due to the use of β-mercaptoethanol (Mommaerts et al. 2015), used to deactivate RNases but with denaturing effects on guanidinium isothiocyanate in the lysis buffer (Kwiatkowski and Kivins 2004). Additionally, the poorer results in Canvax™ and Maxwell® could be attributable to the probable contamination in the eluted *RNA* such as the remains of the magnetic microspheres, interfering in the spectrophotometric quantification, and even in subsequent applications of the *RNA* samples (Stulnig and Amberger 1994; Liu et al. 2009; Martín-Nuñez et al. 2012).

To evaluate the purity of *RNA*, absorbance ratios at and 260 nm/280 nm (DNA/protein) and 260 nm/230 nm (DNA/contaminants) were determined. Accordingly, results showed acceptable 260/280 ratio values ranged from 1.77 to 2.1 for all the methods tested, with exception of Maxwell®, which showed a 260/280 ratio of 4.2 and 6.27 in human Jurkat T cells and PBMC, respectively (Table 1). Only Maxwell® showed significant differences ($P < 0.001$) when was compared with the rest of the methods. The lower 260/230 ratios were obtained with Maxwell® and TRIzol™ methods for human Jurkat T cells, and only using TRIzol™ in PBMC. Best results, among 1.96-2.15, were obtained for QIAcube™, Monarch®, and Canvax™ methods (Table 1). In this respect, lower 260/230 ratios may indicate the presence of compounds absorbing at 230 nm such as proteins (Stulnig and Amberger 1994), guanidine HCL, EDTA, carbohydrates, lipids, salts, or phenol (Psifidi et al. 2015). In comparison with PBMC, the yield obtained in Jurkat T cells was about 5 times higher as corresponding to a more homogeneous matrix with fewer interfering substances than biological fluids.

The analysis of the integrity of *RNA* revealed the presence of both ribosomal 28S and 18S bands, showing the typical 2:1 proportion of intensity, in all *RNA* samples either from human Jurkat T cells (Fig. 1a) or from PBMC (Fig. 1b), irrespective of the method of extraction, as usually reported (Ruettger et al. 2010; Gan et al. 2016), excepting for Maxwell®. In this case, a single very intense band was observed in both biological matrices (Fig. 1), located close to the 28S band but not having correspondence with this.

This could be due to the aforementioned elution of the *RNA* together with the magnetic microspheres used in this method, which might cause changes in the migratory patterns of these *RNA*s in agarose gels (Martín-Núñez et al. 2012; Mommaerts et al. 2015). For 5S band only was observed for Monarch, QIAgen™, QIAcube™, and Bio-Rad methods in human Jurkat T cells, but not for any method in PBMC.

To determine the functionality of *RNA* samples PCR and real-time PCR were performed in genes whose expression level was similar in both human Jurkat T cells and PBMC. All samples were positive for the genes studied, and an amplification band of the desired size was obtained regardless of the type of extraction method and the matrix evaluated (Fig. 1c, d). Multiple comparisons showed significant differences for C_T values in the Bio-Rad, Maxwell®, and TRIzol™ methods (Fig. 1g, h). All *RNA* obtained in our study was shown as functional by conventional PCR and real-time PCR, as observed in related studies (Bayatti et al. 2014; Chauhan et al. 2018). However, statistically significant differences were found in the results of real-time PCR for Bio-Rad, Maxwell®, and TRIzol™ methods when were compared with the rest of the methods tested in both matrices. These results suggest these methods could compromise the functionality of the *RNA*s obtained, so results could not be completely reliable.

Finally, an important fact to consider when comparing nucleic acid extraction methods is to know the time, labour, and cost analysis for each method (Chacon-Cortes et al. 2012; Psifidi et al. 2015). The feasibility of each method in terms of time and costs per sample is reported in table 2. The fastest extraction methods were the Canvax™ and Maxwell®, while the most time-consuming were the QIAgen™, QIAcube™, and manual, which required overnight incubation. On the other hand, the manual was the cheapest one, followed by the Monarch® and Canvax™, two semiautomated methods requiring lower manipulation by specialized personnel than the manual method. Additionally, manual or semiautomated methods can process 24 samples, approximately twice those of automated methods QIAcube™ and Maxwell®. The analysis performed in this study showed that automated methods were quite expensive and had a low capacity to work with several samples simultaneously, while the manual method (TRIzol™) extended the protocol more than one day, increasing the risk of degradation. Therefore, semi-automated methods could be more advisable because they have a more affordable price, a greater capacity than other methods, and less execution time.

In summary, our data revealed differences attributable to the method chosen. Given our results, QIAcube™ and semi-automated extraction methods were perceived as the best options because of a lower variability, good functionality, and lower cost. Noteworthy Monarch® appeared as the second-best option because it showed quality indicators closer to expected, which guarantees reliable results. Despite larger studies with a greater number of methods and matrices would be advisable, the variety of methods compared in this study emphasize the relevance of the choice of an optimal *RNA* extraction method in biomedical and nucleic acid research.

References

1. Aranda R, Dineen S, Craig RL, Guerrieri RA, Robertson JM (2009) Comparison and evaluation of RNA quantification methods using viral, prokaryotic, and eukaryotic RNA over a 10^4 concentration range. Anal Biochem 387:122–127. https://doi.org/10.1016/j.ab.2009.01.003
2. Arraiano CM, Andrade JM, Domingues S, Guinote IB, Malecki M, Matos RG, Moreira RN, Pobre V, Reis FP, Saramago M, Silva IJ, Viegas SC (2010) The critical role of RNA processing and degradation in the control of gene expression. FEMS Microbiol Rev 34:883–923. https://doi.org/10.1111/j.1574-6976.2010.00242.x
3. Bai JP, Alekseyenki AV, Statnikov A, Wang IM, Wong PH (2013) Strategic applications of gene expression: from drug discovery/development to bedside. AAPPS J 15:427–437. https://doi.org/10.1208/s12248-012-9447-1
4. Bayatti N, Cooper-Knock J, Bury JJ, Wyles M, Heath PR, Kirby J, Shaw PJ (2014) Comparison of blood RNA extraction methods used for gene expression profiling in amyotrophic lateral sclerosis. PloS One 9:e87508. https://doi.org/10.1371/journal.pone.0087508
5. Chacon-Cortes D, Haupt LM, Lea RA, Griffiths LR (2012) Comparison of genomic DNA extractions techniques from whole blood samples: a time, cost and quality evaluation study. Mol Biol Rep 39:5961– https://doi.org/10.1007/s11033-011-1408-8
6. Chauhan A, Sharma JN, Modgil M, Siddappa S (2018) Comparison of various RNA extraction methods, cDNA preparation and isolation of calmodulin gene from a highly melanized isolate of apple leaf blotch fungus Marssonina coronaria. J Microbiol Methods 151:7–15. https://doi.org/10.1016/j.mimet.2018.05.023
7. Cooper TA, Wan L, Dreyfuss G (2009) RNA and disease Cell 136:777–793. https://doi.org/10.1016/j.cell.2009.02.011
8. Deng MY, Wang H, Ward GB, Beckham TR, McKenna TS (2005) Comparison of six RNA extraction methods for the detection of classical swine fever by real-time and conventional reverse transcription-PCR. J Vet Diagn Invest 17:574– https://doi.org/10.1177/104063870501700609
9. Desjardins P, Conklin D (2010) NanoDrop Microvolume Quantitation of Nucleic Acids. J Vis Exp 45:2565. https://doi.org/10.3791/2565
10. Ferro P, Ventura R, Pérez-Mañá C, Farré M, Segura J (2016) Genetic and protein biomarkers in blood for the improved detection of GH abuse. J Pharm Biomed Anal 128.111–118. https://doi.org/10.1016/j.jpba.2016.05.022
11. Fleige S, Pfaffl MW (2006) RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med 27:126–139. https://doi.org/10.1016/j.mam.2005.12.003
12. Gan MF, Yang HL, Qian JL, Wu CS, Yuan CX, Li XF, Zou J (2016) Comparison of two methods for RNA extraction from the nucleus pulposus of intervertebral discs. Benet Mol Res 15:gmr.15027738. https://doi.org/10.4238/gmr.15027738
13. García-Nogales P, Serrano A, Secchi S, Gutiérrez S, Arís A (2011) Comparison of commercially-available RNA extraction methods for effective bacterial RNA isolation from milk spiked samples. Electron J Biotechn 13:19– https://doi.org/10.2225/vol13-issue5-fulltext-10

14. Hawkins PG, Morris KV (2008) RNA and transcriptional modulation of gene expression. Cell Cycle 7.602–607. https://doi.org/10.4161/cc.7.5.5522
15. Hernández-Gúzman AK, Guzmán-Barney MM (2013) Comparison of RNA isolation methods for Potato yellow vein virus (PYVV) detection by RT-PCR in different organs of Solanum tuberosum Group Phureja. Rev Colomb Biotecnol 15:71–81. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0123-34752013000100008&lng=en
16. Kwiatkowski RW Jr, Kivins WJ (2004) Archiving DNA: Comparison of Liquid Phase and Magnetic Bead-Based Solid Phase Purification Methods. Gentra Systems Technical Report.
17. Liu PF, Avramova LV, Park C (2009) Revisiting absorbance at 230 nm as a protein unfolding probe. Anal Biochem 389:165–170. https://doi.org/10.1016/j.ab.2009.03.028
18. Liu L, Wang J, Khanabdali R, Kalionis B, Tai X, Xia S (2017) Circular RNAs: Isolation, characterization and their potential role in diseases. RNA Biol 14:1715–1721. https://doi.org/10.1080/15476286.2017.1367886
19. Lucena-Aguilar G, Sánchez-López AM, Barberán-Aceituno C, Carrillo-Ávila JA, López-Guerrero JA, Aguilar-Quesada R (2016) DNA Source Selection for Downstream Applications Based on DNA Quality Indicators Analysis. Biopreserv Biobank 14:264– https://doi.org/10.1089/bio.2015.0064
20. Madera-Anaya MV, Suárez-Causado A (2017) Evaluation of two RNA extraction methods in children's saliva. Rev Odont Mex 21:e237–e243. https://doi.org/10.1016/j.rodmex.2018.01.014
21. Martín-Núñez GM, Gómez-Zumaquero JM, Soriguer F, Morcillo M (2012) High resolution melting curve analysis of DNA samples isolated by different DNA extraction methods. Clin Chim Acta 413:331– https://doi.org/10.1016/j.cca.2011.09.014
22. Martínez B, Jimenez A, Díaz T, Gómez JM, Hortas ML, García-Fuentes E, Segura J, Ferro P (2018) Development of COL3A1-Specific Real-Time PCR Assay for Clinical and Sport Detection Applications. J Chromatogr Sep Tech 9:403. https://doi.org/10.4172/2157-7064.1000403
23. Mihailescu R (2015) Gene expression regulation: lessons from noncoding RNAs. RNA 21.695– https://doi.org/ 10.1261/rna.050815.115
24. Mommaerts K, Sanchez I, Betsou F, Mathieson W (2015) Replacing beta-mercaptoethanol in RNA extractions. Anal Biochem 479:51–53. https://doi.org/10.1016/j.ab.2015.03.027
25. Oliveira-Alves MG, Pérez-Sayáns M, Padín-Iruegas ME, Reboiras-López MD, Suarez-Peñaranda JM, López-López R, Carta CF, Issa JS, García-García A, Almeida JD (2016) Comparison of RNA extraction methods for molecular analysis of oral cytology. Acta Stomatol Croat 50:108–115. https://doi.org/10.15644/asc50/2/2
26. Optiz L, Salinas-Riester G, Grade M, Jung K, Emons PJoG, Ghadimi BM, Beissbarth T, Gaedke J (2010) Impact of RNA degradation on gene expression profiling. BMC Med Genomics 9:6. https://doi.org/10.1186/1755-8794-3-36
27. Ortega-Pinazo J, Díaz T, Martínez B, Jiménez A, Pinto-Medel MJ, Ferro P (2019) Quality assessment on the long-term cryopreservation and nucleic acids extraction processes implemented in the

andalusian public biobank. Cell Tissue Bank 20:255–265. https://doi.org/10.1007/s10561-019-09764-9

28. Psifidi A, Dovas CL, Bramis G, Lazou T, Russel CL, Arsenos G, Banos G (2015) Comparison of Eleven Methods for Genomic DNA Extraction Suitable for Large-Scale Whole-Genome Genotyping and Long-Term DNA Banking Using Blood Samples. PLoS One e0115960. https://doi.org/10.1371/journal.pone.0115960
29. Reimann E, Abram K, Kõks S, Kingo K, Fazeli A (2019) Identification of an optimal method for extracting RNA from human skin biopsy, using domestic pig as a model system. Sci Rep 9:20111. https://doi.org/10.1038/s41598-019-56579-5
30. Ruettger A, Neumann S, Wiederanders B, Huber R (2010) Comparison of different methods for preparation and characterization of total RNA from cartilage samples to uncover osteoarthritis in vivo. BMC Res Notes 3, 7. https://doi.org/10.1186/1756-0500-3-7
31. Stulnig TM, Amberger A (1994) Exposing contaminating phenol in nucleic acid preparations. Biotechniques 16:402–404.
32. Tavares L, Alves PM, Ferreira RB, Santos CN (2011) Comparison of different methods for DNA-free RNA isolation from SK-N-MC neuroblastoma. BMC Res Notes 4:3. https://doi.org/10.1186/1756-0500-4-3
33. Thatcher SA (2015) DNA/RNA preparation for molecular detection. Clin Chem 61:89–99. https://doi.org/10.1373/clinchem.2014.221374
34. Umaña R, Pritschl C, Arbiza JR, Rivas F, Pagliano G (2013) Evaluation of four viroid RNA extraction methods for the molecular diagnosis of CEVd in Citrus lemon using RT-PCR, Dot blot and Northern blot. Biotecnol Apl 30:131–136. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1027-28522013000200006&lng=es&tlng=en.
35. Zhang C (2009) Novel functions for small RNA molecules. Curr Opin Mol Ther 11:641–651.

Tables

Table 1. Comparative values of spectrophotometry (Nanodrop™) and fluorimetry (QUBIT™) (expressed as mean ± SD), 260/280 and 260/230 ratios in *RNA* obtained from human Jurkat T cells (HJT) and PBMC using seven different extraction methods.

kit		NANODROP ™2000			QUBIT™
		µg Mean±SD	260/280 ratio	260/230 ratio	µg Mean±SD
QIAgen™	*HJT*	27.61 ± 0.76	2.1 ± 0	1.47 ± 0.17	25.24 ± 0.22
	PBMC	4.95 ± 1.91	1.89 ± 0.11	1.10 ± 0.34	3.93 ± 1.7
Bio-Rad	*HJT*	8.89 ± 2.14	2.1 ±0.01	1.56 ± 0.40	8.05 ± 1.95
	PBMC	3.29 ± 1.28	2 ±0.04	1.38 ± 0.39	2.66 ± 0.79
Monarch®	*HJT*	17.88 ± 2.48	2.1 ± 0.02	2.15 ± 0.03	16.30 ± 1.68
	PBMC	4.02 ± 0.97	2.02 ± 0.01	2.03 ± 0.14	3.41 ± 0.87
Canvax™	*HJT*	4.51 ± 0.94	2.1 ± 0	1.96 ± 0.07	4.06 ± 0.53
	PBMC	--	--	--	--
Maxwell®	*HJT*	2.99 ±1.04	4.2±0.58	0.64 ± 0.41	2.49 ± 0.56
	PBMC	2.66 ± 0.6	6.27±1.06	1.13 ± 0.29	1.64 ± 0.45
QIAcube™	*HJT*	33.47 ± 3.77	2.1 ± 0.01	2.056 ± 0.41	31.02 ± 4.56
	PBMC	6.13 ± 2.98	1.98 ± 0.10	1.58 ± 0.31	4.84 ± 1.9
TRIzol™	*HJT*	22.27 ± 5.32	1.9 ± 0.04	0.96 ± 0.15	20.28 ± 4.17
	PBMC	7.22 ± 3.5	1.77 ± 0.12	0.54 ± 0.21	5.76 ± 2.2

Table 2. Assessment of consumables cost per sample and process duration *RNA* extraction methods studied.

	QIAgen™	Bio-Rad	Monarch®	Canvax™	QIAcube™	Maxwell®	TRIzol™
Cost per sample (USD)	7.0	5.91	4.82	5.25	7.0	10.18	4.43
ocess duration (10 samples)	70 min	60 min	90 min	45 min	70 min	45 min	100 min
Overnight incubation	Yes	No	No	No	Yes	No	Yes
aximum capacity (samples)	24	24	24	24	12	16	24

Figures

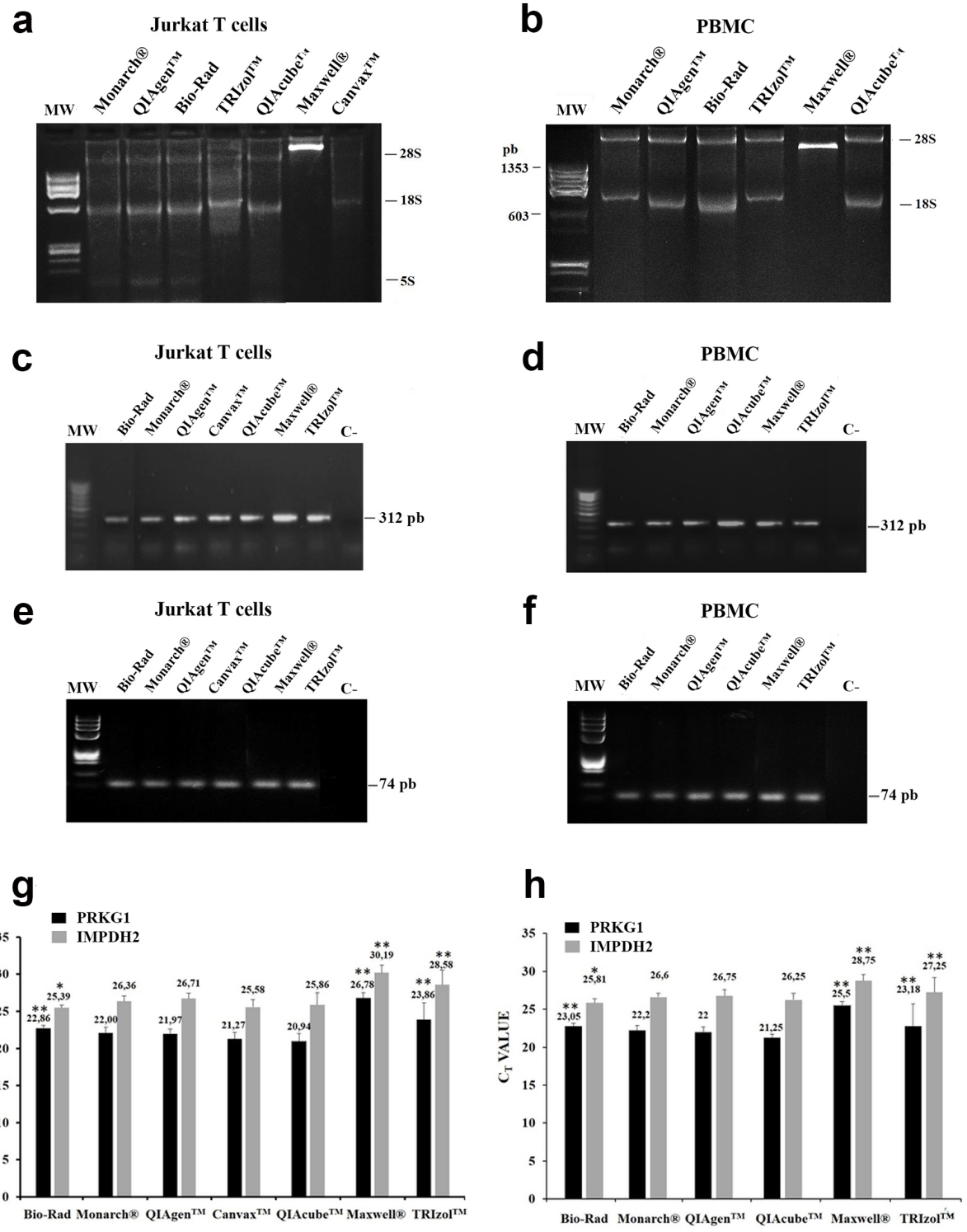

Figure 1

RNA analysis in human Jurkat T cells and PBMC. (a, b) RNA integrity in samples processed with different extraction methods in human Jurkat T cells (a) and PBMC (b) in 3% agarose gels. MW: Molecular weight marker (0.072-1.35 kbp). (c-f) Assessment of functionality of RNA obtained in human Jurkat T cells and PBMC; HIST1H4A gene fragment (312 bp) amplified from cDNA in 2% agarose gels by conventional PCR (c, d); PRKG1 fragment gene (74 bp) amplified from cDNA in 2% agarose gels by real-time PCR (e, f); MW:

Molecular weight marker (0.072-1.35 kbp), C-: Negative control. (g, h) RNA performance for real-time PCR assay in human Jurkat T cells and PBMC for PRKG1 (black) and IMPDH2 (gray) genes. The average CT value and standard deviation for each type of source were calculated. * $0.01 < p \leq 0.05$ and ** $0.001 < p \leq 0.01$