**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 and Biotechnology. Because of their 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 or in research service platforms such as biobanks to see the traceability of the samples. In this study, we have compared seven different RNA extraction methods: manual (TRIzol™), semiautomated (QIAGEN™, Bio-Rad, Monarch®, and Canvax™), and fully automated (QIAcube™ and Maxwell®) processes, 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. QIAcube™ and semi-automated extraction methods were perceived as the best options because of their lower variability, good functionality, and lower cost ($P < 0.001$). These data contribute to facilitating researchers or research service platforms (Biobanks) in decision-making practices and emphasize the relevance of the selection of the RNA extraction method in each experimental procedure or traceability study to guarantee both quality standards and its reproducibility.

Keywords: RNA, RNA Extractions Methods, PBMC, Jurkat T cell, PCR, real-time PCR.

1. Introduction

Nucleic acids are widely used in biomedical and biotechnology research and clinical practice. Specifically, ribonucleic acid (RNA), which is found in every living cell and is involved in multiple functions, due to the large existing RNA variety [1-3]. Thus mRNA, RNAi, small RNA, and microRNA (among others) have been key to improving our understanding of gene expression control for the study, diagnosis, and treatment in many different areas of pathology [4-8]. Because RNA degradation is critical [9, 10] 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 [11].

Currently, there are several indicators of RNA quality. For RNA purity, spectrophotometry by evaluating the A260/A280 and A260/A230 ratios or fluorimetry using the Quant-iT™ RiBogreen® RNA can be used [12-14]. 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 [15]. 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 [16]. Obtaining RNAs with optimal values for all these indicators is essential to generate reliable results.

The 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 [17-20] and matrices, including saliva, whole blood, and skin [21-25], revealed differences in the yield, quality, and functionality of RNA obtained. However, at present no studies are comparing a substantial number of RNA extraction methods, especially, using the so extensively studied peripheral blood mononuclear cells (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®) processes. RNA samples were evaluated by spectrophotometry and fluorimetry to determine their yield and purity.

Subsequently, an assessment of integrity and a functionality study were carried out. Finally, a determination of cost, process time, and other related factors was 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 the validity of laboratory RNA methods, according to their requirements.

2. Materials and Methods

2.1. 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 penicillin-streptomycin 1x (all products from Merck Life Science SLU, Spain) at 37 °C in a humid atmosphere of 5% CO₂. Confluent cell cultures were centrifuged to harvest cells and washed in Dulbecco phosphate-buffered saline (DPBS) without calcium or magnesium. After centrifugation, cells were cryopreserved in RPMI supplemented with 40% FBS and 10% dimethyl sulfoxide (DMSO) and stored at -196 °C until use in aliquots of 3.5×10⁶ cells. Cell cultures analyzes were performed in triplicates.

2.2. Subjects

Blood samples were obtained from 50 volunteers, collected in 4 ml EDTA BD Vacutainer™ tubes (Becton Dickinson & Company, New Jersey, USA). After processing the samples to obtain PBMC from each, there were stored at -196 °C 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).

2.3. PBMC purification

Blood samples were obtained from 50 volunteers, collected in 4 mL EDTA BD Vacutainer™ tubes (Becton Dickinson & Company, New Jersey, USA). After processing the samples to obtain PBMC from each, there were stored at -196 °C 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). The procedure was carried out as previously described by Ortega-Pinazo et al. [26]. Briefly, Lymphosep™ (Biowest, Riverside, Missouri, USA) was added to a sterile tube, and 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 continuously at room temperature. PBMC layer was collected and transferred into a new tube. Then, two washing steps with physiological saline at 4°C 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 3.5×10⁶ cells were cryopreserved in RPMI supplemented with 40% FBS and 10% DMSO and stored at -196°C.

2.4. RNA extraction

Jurkat-t and PBMCs cells were thawed and washed twice with DPBS without calcium or magnesium at 122 ×g for 8 min to remove DMSO. Then, pellets of 3×10⁶ viable cells were used for RNA extraction 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 °C 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, Pellet was resuspended in 700 µL of TRIzol™ (Invitrogen, Thermo Fisher Scientific, Massachusetts, USA) and stored at -80 °C 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.

1 - **QIAGEN™**: Previously, Pellet was resuspended in 700 µL of TRIzol™ and stored at
2 -80 °C for 48 hours. RNA was manually extracted from samples with the commercial
3 QIAmp™ RNeasy mini Kit (QIAGEN™), following the manufacturer's instructions.

4
5 - **Bio-Rad**: RNA was extracted with the commercial Aurum™ Total RNA Mini Kit (Bio-
6 Rad, California, USA), following the manufacturer's instructions.

7
8 - **Monarch®**: RNA was extracted with the commercial Monarch® Total RNA Miniprep
9 Kit (New England Biolabs, Massachusetts, USA), following the manufacturer's
10 instructions.

11
12 - **Canvax™**: RNA was extracted with the commercial HigherPurity™ Total RNA
13 Extraction Kit (Canvax™ Biotech S.L., Córdoba, Spain), following the manufacturer's
14 instructions.

15
16 - **TRIzol™**: The cell pellet was incubated with 800 µL of TRIzol™ (Invitrogen) at RT
17 for 10 min with agitation. Then, 200 µL of chloroform was added and. after manual
18 agitation for 15 seconds, the samples were then cold centrifuged at 12000 ×g for 15
19 min. The transparent phase was collected for RNA extraction and transferred to a new
20 sterile tube adding 600 µL of isopropanol. After 20 min incubation at -20 °C, samples
21 were cold centrifuged at 12000 ×g for 10 min. The pellet was washed once with 1 ml
22 of ethanol 75%. After cold centrifugation at 7500 ×g for 5 min, the pellet was dried to
23 remove the excess ethanol.

24 **2.5. RNA quantification, purity, and integrity**

25 Absorbance at 260, 280, and 230 nm of 2 µL of each RNA sample was
26 measured in duplicate using the NanoDrop™ 2000 (ThermoFisher Scientific).
27 Ultrapure water was used as blank. The concentration of RNA from 260 nm
28 absorbance was calculated according to the Lambert-Beer law. The A260/A280 ratio
29 (i.e., RNA/protein) was used as a purity indicator. Optimum A260/A280 ratio values or
30 pure RNA ranged from 1.8 to 2. The A260/A230 ratio (RNA/contaminants) was used
31 as a secondary measurement of purity, establishing the optimal, in this case, at values
32 greater than 1.8 [27]. The concentration of RNA was also performed using a
33 fluorometric method. Thus, Qubit™ RNA HS Assay Kit (Life Technologies, Thermo

Fisher Scientific) was used following the manufacturer's instructions. First, standard samples 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).

2.6. RNA functionality

RNA functionality was evaluated by PCR amplification or real-time PCR assay of commonly used reference genes, available and frequently studied in our laboratory. To obtain the cDNA, 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 -20°C. For PCR, a fragment of 312 base pairs of the HIST1H4A gene was amplified following the protocol previously published by Ortega-Pinazo et al. [26]. For the real-time PCR assay, 25 ng of cDNA from each sample was amplified for the *PRKG1* and *IMPDH2* genes obtaining a PCR product of 74 and 98 bp respectively and delta Ct value was compared for each extraction method, in an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystem, California, USA) using iTaq™ Universal SYBR Green Supermix (Bio-Rad). Determinations were performed in triplicate, and the C_T value was analyzed. 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).

2.7. 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.

3. Results and discussion

Yield and quantification studies for each extraction method were initially carried out in cell cultures (Jurkat T cells) due to the homogeneity of the samples, allowing an optimal comparison between tested methods. 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 the 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 a significantly ($P < 0.001$) higher performance in the RNA samples obtained with TRIzol™, QIAgen™, or QIAcube™ methods, and 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 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.

Furthermore, the long-term storage of nucleic acids is needed in biomedical research to develop research lines based on previous results that allow the same samples to be studied over several years. Some studies on RNA storage for long periods have been carried out, but these have been carried out at temperatures about $-80\text{ }^{\circ}\text{C}$ or in blood samples preserved in Tempus tubes [28, 29]. Results obtained show that the RNAs obtained are viable 3 years after their storage at $-26\text{ }^{\circ}\text{C}$, watching a

1 slight decrease in yield after quantification but presenting integrity values identical to
2 those initially obtained (Table 3). It should be noted that the only significant differences
3 were found for the manual method, perhaps because the reagents used in the
4 extraction produced a partial degradation in the sample. The functionality studies were
5 similar to those obtained initially.

6 From these findings, the best yield was obtained for QIAcube™ (automated),
7 QIAgen™ (semi-automated), and TRIzol™ (manual) methods. In line with our results
8 from Jurkat T cells, a previous work from Tavares et al. [30] using SK-N-MC
9 neuroblastoma cells reported that semi-automated methods had better yield than
10 manual ones. However, for PBMC, a yield up to 1.18 times higher was found in manual
11 extraction methods when compared with commercial kits [18,21], in agreement with
12 our study. The decrease in the yield of some semi-automated methods, such as Bio-
13 Rad and Canvax™, may be due to the use of β -mercaptoethanol, used to deactivate
14 RNases but with denaturing effects on guanidinium isothiocyanate in the lysis buffer
15 [31]. Additionally, the poorer results in Canvax™ and Maxwell® could be attributable
16 to the probable contamination in the eluted RNA such as the remains of the magnetic
17 microspheres, interfering in the spectrophotometric quantification, and even in
18 subsequent applications of the RNA samples [32-34].

19 To evaluate the purity of RNA, A260/A280 (DNA/protein) and A260/A230
20 (DNA/contaminants) absorbance ratios were determined. Accordingly, results showed
21 acceptable A260/A280 ratio values ranging from 1.77 to 2.1 for all the methods tested,
22 with exception of Maxwell®, which showed an A260/A280 ratio of 4.2 and 6.27 in
23 human Jurkat T cells and PBMC, respectively (Table 1). Only Maxwell® showed
24 significant differences ($P < 0.001$) when compared with the rest of the methods. The
25 lower A260/A230 ratios were obtained with Maxwell® and TRIzol™ methods for
26 human Jurkat T cells, and only using TRIzol™ in PBMC. Best results, among 1.96-
27 2.15, were obtained for QIAcube™, Monarch®, and Canvax™ methods (Table 1),
28 being this result is similar to that obtained by Sharma P et al. (35) in pediatric PBMCs
29 using the QIAcube™ method. In this respect, lower A260/A230 ratios may indicate the
30 presence of compounds absorbing at 230 nm such as proteins [33], guanidine HCL,
31 EDTA, carbohydrates, lipids, salts, or phenol [36]. Compared with PBMC, the yield
32 obtained in Jurkat T cells was about 5 times higher as would befit to a more
33 homogeneous matrix with fewer interfering substances than biological fluids.

1 The analysis of the integrity of RNA revealed the presence of both ribosomal
2 28S and 18S bands, showing the typical 2:1 proportion of intensity, in all RNA samples
3 either from human Jurkat T cells (Fig. 1a) or from PBMC (Fig. 1b), irrespective of the
4 method of extraction, as usually reported [20,21], excepting for Maxwell®. In this case,
5 a single very intense band was observed in both biological matrices (Fig. 1), located
6 close to the 28S band but not having correspondence with this. This could be due to
7 the aforementioned elution of the RNA together with the magnetic microspheres used
8 in this method, which might cause changes in the migratory patterns of these RNAs in
9 agarose gels [29,34]. For 5S band only was observed for Monarch, QIAgen™,
10 QIAcube™, and Bio-Rad methods in human Jurkat T cells, but not for any method in
11 PBMC.

12 To determine the functionality of RNA samples PCR and real-time PCR were
13 performed in genes whose expression level was similar in both human Jurkat T cells
14 and PBMC. All samples were positive for the genes studied, and an amplification band
15 of the desired size was obtained regardless of the type of extraction method and the
16 matrix evaluated (Fig. 1c, d). Multiple comparisons showed significant differences in
17 C_T values in the Bio-Rad, Maxwell®, and TRIzol™ methods (Fig. 1g, h). All RNA
18 obtained in our study was shown as functional by conventional PCR and real-time
19 PCR, as observed in related studies [19,23]. However, statistically significant
20 differences were found in the results of real-time PCR for Bio-Rad, Maxwell®, and
21 TRIzol™ methods when compared with the rest of the methods tested in both
22 matrices. These results suggest these methods could compromise the functionality of
23 the RNAs obtained, so the results could not be completely reliable.

24 Finally, an important fact to consider when comparing nucleic acid extraction
25 methods is to know the time, labor, and cost analysis for each method [36,37]. The
26 feasibility of each method in terms of time and costs per sample is reported in table 2.
27 The fastest extraction methods were the Canvax™ and Maxwell®, while the most
28 time-consuming were the QIAgen™, QIAcube™, and manual, which required
29 overnight incubation. On the other hand, the manual was the cheapest one, followed
30 by the Monarch® and Canvax™, two semiautomated methods requiring lower
31 manipulation by specialized personnel than the manual method. Additionally, manual
32 or semiautomated methods can process 24 samples, approximately twice those of
33 automated methods QIAcube™ and Maxwell®. The analysis performed in this study
34 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 by 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.

4. Conclusion

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 their 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. In addition, RNA stored for 3 years at -26°C showed that they maintained their purity and functionality, which would be very useful for optimizing sample storage in research platforms such as biobanks.

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 SSPA Biobank 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 is not applicable to this article as no datasets were generated or analyzed during the current study.

Author Disclosure Statement

The authors have no conflicts of interest to declare.

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12 Figure Captions

14 **Fig. 1** RNA analysis in human Jurkat T cells and PBMC. **(a, b)** RNA integrity in
15 samples processed with different extraction methods in human Jurkat T cells **(a)** and
16 PBMC **(b)** in 3% agarose gels. MW: Molecular weight marker (0.072-1.35 kbp). **(c-f)**
17 Assessment of functionality of RNA obtained in human Jurkat T cells and PBMC;
18 HIST1H4A gene fragment (312 bp) amplified from *cDNA* in 2% agarose gels by
19 conventional PCR **(c, d)**; PRKG1 fragment gene (74 bp) amplified from *cDNA* in 2%
20 agarose gels by real-time PCR **(e, f)**; MW: Molecular weight marker (0.072-1.35 kbp),
21 C-: Negative control. **(g, h)** RNA performance for real-time PCR assay in human Jurkat
22 T cells and PBMC for PRKG1 (black) and IMPDH2 (gray) genes. The average C_T value
23 and standard deviation for each type of source were calculated. * $0.01 < p \leq 0.05$ and
24 ** $0.001 < p \leq 0.01$

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	A260/A280 ratio	A260/A230 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)	15.75	14.63	14.09	11.81	17.75	18.89	10.45
Process 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
Maximum capacity (samples)	24	24	24	24	12	16	24

Table 3. Mean value of spectrophotometry (Nanodrop™) and fluorimetry (QUBIT™) in *RNA* stored for 2 years and obtained from human Jurkat T cells (HJT) using seven different extraction methods.

kit	2019		2021	
	NANODROP™2000	QUBIT™	NANODROP™2000	QUBIT™
QIAgen™	27.61 ± 0.76	25.24 ± 0.22	28.32 ± 1.85	24,86 ± 1.34
Bio-Rad	8.89 ± 2.14	8.05 ± 1.95	7.25 ± 2.24	6,57 ± 2.5
Monarch®	17.88 ± 2.48	16.30 ± 1.68	16.08 ± 6.05	14.25 ± 5.47
Canvax™	4.51 ± 0.94	4.06 ± 0.53	3.70 ± 1.7	3.55 ± 1.01
Maxwell®	2.99 ±1.04	2.49 ± 0.56	1.52 ± 0.81	2,1 ± 0,09
QIAcube™	33.47 ± 3.77	31.02 ± 4.56	29.25 ± 2.56	24,86 ± 2.16
TRIzol™	22.27 ± 5.32	20.28 ± 4.17	15.07 ± 4.73	12,07 ± 0,29