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Systematic comparison of quantity and quality of RNA recovered with commercial FFPE tissue extraction kits

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Abstract

Background FFPE tissue samples are commonly used in biomedical research and are a valuable source for next-generation sequencing in oncology, however, extracting RNA from these samples can be difficult the quantity and quality achieved can impact the downstream analysis. This study compared the effectiveness of seven different commercially available RNA extraction kits specifically designed for use with FFPE samples in terms of the quantity and quality of RNA recovered.

Methods This study used 9 samples of FFPE tissue from three different types of tissue (Tonsil, Appendix and lymph node of B-cell lymphoma) to evaluate RNA extraction methods. Three sections of 20 µm of each sample were combined per sample. The slices were distributed in a systematic manner to prevent any biases. Each of the 7 commercially available RNA extraction kits were used according to manufacturer's instructions, with each sample being tested in triplicate resulting in a total of 189 extractions. The concentration, RNA quality score (RQS) and DV200 of each extraction was analysed using a nucleic acid analyser to determine the quantity and quality of the recovered RNA.

Results This study found that despite processing the FFPE samples in the same standardized way, there were disparities in the quantity and quality of RNA recovered across the different tissue types. Additionally, the study found notable differences in the quantity of RNA recovered when using different extraction kits. In terms of quality, three of the kits performed better than the other four in terms of RQS and DV200 values.

Conclusion Though many laboratories have developed their own protocols for specific tissue types, using commercially available kits is still a popular option. Although these kits use similar processes and extraction procedures, the amount and quality of RNA obtained can vary greatly between kits. In this study, among the kits tested, while the Roche kit, provided a nearly systematic better-quality recovery than other kits, the ReliaPrep FFPE Total RNA miniprep from Promega yielded the best ratio of both quantity and quality on the tested tissue samples.

Keywords FFPE RNA extraction kit, RNA quality, RNA quantity

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Introduction

FFPE samples, which are created by preserving biopsies or autopsy samples in formalin and embedding them in paraffin, are one of the most valuable resources for biomedical research. There are over a billion FFPE samples stored in hospitals and tissue banks worldwide [1, 2]. This method of preservation is preferred for a variety of reasons, first and foremost that it maintains the original tissue's morphological features well for diagnostic purposes and is more cost-effective and simpler to process and store than fresh frozen samples [3]. Additionally, while the proteins, DNA and RNA are of better quality in fresh frozen tissue, the long-term preservation of the sample can still lead to RNA and protein degradation even at − 80 °C or liquid nitrogen over long period of time. For obvious logistical and economical reasons long storage of FFPE samples was always favoured in many institutions leading to large scale biobank stored in FFPE [4].

RNA extraction from formalin-fixed paraffin-embedded (FFPE) tissue samples can be challenging as the quality of RNA can get affected by the oxidation, crosslinking, and other chemical modifications occurring during the fixation and preservation process, which can cause extensive damage to the RNA. RNA was first extracted from FFPE samples in 1988 [5], and since then it has been commonly used for real-time polymerase chain reaction (RT-PCR) protocols. With the advancement of next-generation sequencing (NGS), specifically RNA sequencing (RNASeq), it has become a powerful tool for both research and potential clinical use. Despite the greater instability of RNA compared to DNA, many studies have found a high correlation between RNASeq data from fresh frozen and FFPE samples [2, 6, 7]. However, extracting high-quality RNA from FFPE samples remains a challenge, and the low quantity or poor quality of RNA recovered can negatively impact the reliability of RNASeq data.

Recent advances in technology have led to the development of methods that can effectively extract high-quality RNA from FFPE samples. One such method is the use of specific enzymes and buffers that can degrade the crosslinks formed by formalin fixation and release the RNA. For instance, proteinase K is a widely used enzyme that digests proteins and can assist in breaking down the crosslinks formed by formalin fixation [8]. Another approach, Heat-Induced Epitope Retrieval (HIER), involves heating the sample in a buffer, such as citrate or Tris–EDTA, to help reverse formalin crosslinks [9, 10]. Additionally, buffers like sodium borohydride can break formaldehyde crosslinks by reducing Schiff bases formed during fixation [11]. Additionally, buffers like sodium borohydride can break formaldehyde crosslinks by reducing Schiff bases formed during fixation.

Many proprietary mixtures, referred to as "lysis buffers," combine these enzymes and buffers, as evaluated in our study. Alternative proprietary mixes, such as the truXTRAC kit by Covaris, utilize specific enzymes and buffers to degrade crosslinks formed by formalin fixation. Kresse et al. demonstrated that this method provides high yields and quality RNA suitable for downstream applications like RNA-seq [12]. Similarly, the Agencourt FormaPure Kit by Illumina, another enzyme-based method, has shown high efficiency in extracting RNA from FFPE samples, particularly for detecting fusion genes in high-throughput sequencing; however, this kit was not evaluated in our study. Amini et al. utilized laser capture microdissection (LCM) to physically isolate specific regions of tissue containing the target RNA prior to extraction. Their approach involved focused ultrasonication, which enabled RNA isolation from small amounts of material with a higher yield compared to protease-based extraction methods [13]. Additionally, many manufacturers have developed kits for extracting RNA from FFPE samples, making the process more straightforward and replicable. However, these kits can vary greatly in their efficiency for different tissue types in terms of the quantity and quality of RNA recovered. To evaluate this variation, in this study, we systematically tested the quantity and quality of RNA recovered with seven commercial kits for manual extraction, using identical samples from three different tissue types.

Material and methods

Tissue preparation, sectioning, and storage

Samples from three tonsils from individuals with tonsillitis, three appendixes from individuals with appendix inflammation, and three lymph nodes from patients affected by B-cell lymphoma were surgically removed at Sidra Medicine hospital. After surgery, the samples were processed by the Pathology department of Sidra Medicine hospital for diagnostic purposes following their standard operating procedures. Briefly, the samples were cut into approximate $0.5 \times 1 \times 1$ cm blocks and incubated in 10% neutral buffered formalin (Diapath, #F0045) for 18 to 48 h. The samples were then paraffin-embedded using a Leica EG1150H (Leica) and stored at room temperature not exceeding 25 °C. 5 µm slices were made and H&E stained for diagnostic purposes. All blocks used were relatively homogenous in tissue and tissue composition.

After several months of storage (6–12 months) at room temperature in the pathology lab archive, the samples were designated as diagnostic surplus and processed for RNA extraction.

To avoid regional biases of cell type or abundance within each block, slices were systematically distributed across the sample collection tubes. Specifically, 20 µm

thick slices were cut from the block, distributed to 27 tubes with 3 slices per tube, and then stored at − 80 °C until RNA extraction (less than 1 month). As illustrated in Fig. 1, a total of 81 slices of 20 µm each were cut and distributed among 27 tubes, with each tube receiving one slice from every 27 cuts and avoiding consecutive slices in the same tube.

RNA extraction

RNA extraction was performed using each kit listed below according to manufacturer specific recommendations (Table 1). When de-paraffinization solution was not included in the kit, Xylene was used as recommended by the manufacturer. When the kit was proposing a range of elution volume, the minimum volume was chosen in each case. Each kit extraction was performed in separate days by the same operator.

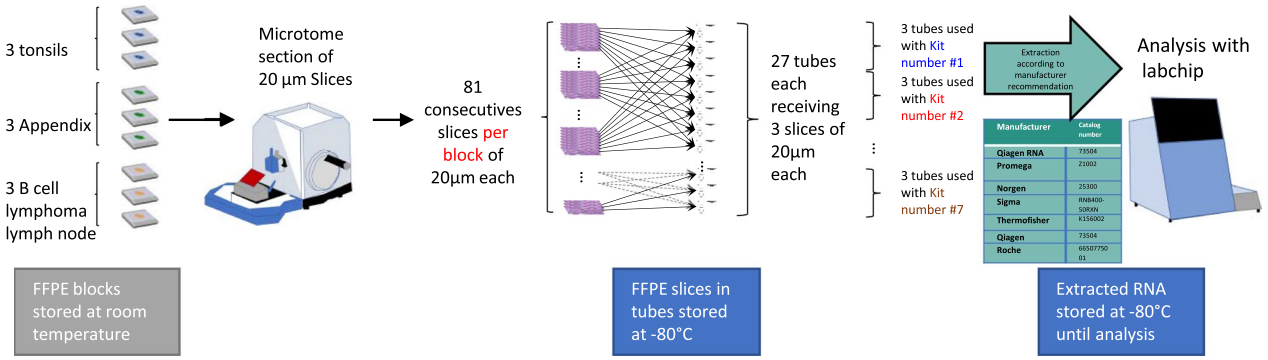

Fig. 1 Flow chart of tissue sampling. Three independent samples of three types of FFPE tissue (tonsil, appendix and lymph node from B-cell lymphoma) were used in the study, resulting in a total of nine samples. The samples were processed in the pathology lab at Sidra medicine hospital following standard operating procedures. The samples were fixed in formalin for 19–48 h, embedded in paraffin, and stored at room temperature as FFPE blocks. Each block was then cut into 20 µm sections using a microtome (Leica, #RM2255 Rotary). To avoid regional biases in the tissue sample, a total of 81 sections were cut from each block and distributed systematically across 27 collection tubes. Each tube received three sections of tissue, with one section taken from every other 27 sections. The tubes were then stored at − 80 °C until extraction with each kit. Each tissue sample was extracted in triplicate using each kit (referred to here as Kit#1, Kit#2, etc., and listed in Table 1), for a total of 27 extractions per kit and a total of 189 extractions. The extracted RNA was stored at − 80 °C and quantitative and qualitative analysis were performed simultaneously using the LabChip® GX Touch™ nucleic acid analyser

Table 1 List of kit used with catalogue number and number of test included in each kit, price per kit and per test and hands-on time requirement

Manufacturer	Kit name	Catalogue number	Number of extractions with 1 kit	Cost per kit (USD)	Cost per extraction	Approximative total hands-on time (in minutes) (incubation time indicated)
Qiagen (W)	RNeasy FFPE Kit	73504	50 samples	632	12.64	110 min hands-on + 2X15min incubation
Promega (W)	ReliaPrep™ FFPE Total RNA Miniprep System	Z1002	100 samples	949	9.49	110 min hands-on + 15 min + 60 min + 15 min incubation
Norgen (W)	FFPE RNA Purification Kit	25300	50 samples	435	8.7	105 min hands-on + 10 min + 2X15min incubation
Sigma	GenElute™ FFPE RNA Purification Kit	RNB400-50RXN	50 samples	806	16.12	100 min + 10 min + 2X15min incubation
Thermofisher (W)	PureLink™ FFPE RNA Isolation Kit	K156002	50 samples	427	8.54	100 min hands-on + 10 min + 60 min incubation
Qiagen (W)	AllPrep DNA/RNA FFPE Kit	80234	50 samples	950	19	120 min hands-on + 2X15min incubation
Roche (W)	High Pure FFPET RNA Isolation Kit	6650775001	50 samples	395	7.9	100 min hands-on + 15 min + 10 min + 60 min incubation

All kits rely on similar sequential steps starting from deparaffinisation of sample, digestion, binding to column, cleaning and finally elution. Yet they all use proprietary "buffers" and solution making difficult the chemical comparison of those kits. Also while some include deparaffinization solution (like oil) some offer the choice of third party product to be used. In our test all offered the possibility to use Xylene, therefore when not provided the deparaffinization was performed using Xylene. Also the manufacturer usually offer the possibility of elution in a range of volume. As in some post extraction procedure a concentration can be essential, we decided to test each kit using their minimum required elution volume (Table 2). Finally, we indicated in Table 2 the main temperature and incubation time after deparaffinization as this can be crucial for quality of RNA recovered as discussed below.

Certain kits required additional reagents, such as ethanol and xylene, which must be supplied by the end user. The quality of these reagents can influence the quality of the extracted RNA; in our case, when needed, we used 200-proof ethanol (Sigma #E7023). Other standard laboratory consumables, such as nuclease-free tips and tubes, were also required by the end user for all kits.

RNA analysis using nucleic acid analyser

RNA quantity was evaluated with 2 instruments spectrophotometry using Nanodrop 8000 (Thermo fisher scientific, # ND-8000-GL). The quantity and quality was also assessed using RNA reagent kits (# CLS960010) and RNA labchips (# 760435) were obtained from Perkin Elmer (Waltham, MA). Nucleic acid analysis of sample were performed according to manufacturer specific recommendations. All data obtained are summarised in supplementary Table 1. Concentration reading from the nucleic acid analyser were used for analysis.

Statistics

Shapiro–Wilk test showed that the data were not normally distributed, we therefore used Wilcoxon Signed-Rank Test to determine statistical differences between the extractions of the same samples and across samples between kits. We also used one-way ANOVA to compare differences among groups.

Results

Quantitative analysis

Using nucleic acid analyser measurements, the quantity of RNA recovered with each kit was first analysed. To account for variability in tissue processing and tissue type that could affect recovery efficiency, the relative quantity was analysed based on the best-performing kit for each sample. For all 3 tonsils and all 3 lymph nodes samples from B-cell lymphoma patients and for one appendix sample, the Promega kit provided the maximum recovery, while for two appendix samples, the kit from Thermofisher performed better than the other kits (Fig. 2A–C). Overall, the Promega kit allowed significantly higher quantity recovery than the other kits (Fig. 2D).

Qualitative analysis

The nucleic acid analyser system performed two different quality analyses, RQS (on a scale of 1 to 10) and DV200 (expressed in percentage). The RQS (RNA quality score) is a parameter to assess the integrity of RNA. The RQS value is derived by the size distribution of the RNA and it represents the degree of integrity/degradation of a given sample, with a score of 10 corresponding to intact RNA and a score of 1 corresponding to a highly degraded RNA. The DV200 is a measure of RNA quality representing the percentage of RNA fragments > 200 nt. First, these two-quality metrics were analysed separately. When analysing RQS values, the Thermofisher kit performed significantly better than the other kits for Tonsils 1 and 2, but for Tonsil 3. Promega, Thermofisher, and Roche kits had no significantly different result on Tonsils, but all three performed better than the other kits (Fig. 3A). For Appendixes, Thermofisher and Roche kits performed better than the other kits for appendix 1 and 2, and the Roche kit performed better than any other kit for appendix 3

Table 2 Deparaffinization solution used, elution volume and incubation time and temperature used for each kit

Kit	Deparaffinization solution provided	Deparaffinization solution used	Elution volume recommended	Volume of elution done	Temperature and time of incubation for deparaffinization (°C)
Qiagen FFPE RNA	Yes	Provided	14–30	14	56 °C (15 min) 80 °C (15 min)
Promega	Yes	Provided	30–50	30	56 °C (15 min) 80 °C (1 h)
Noragen	No	Xylene	20–50	20	55 °C (15 min) 80 °C (15 min)
Sigma	No	Xylene	20–50	20	55 °C (15 min) 80 °C (15 min)
Thermo	Yes	Provided	50	50	60 °C (10 min) 72 °C (1 h)
Qiagen ALL prep	Yes	Provided	14–30	14	56 °C (15 min) 80 °C (15 min)
Roche	No	Xylene	25	25	55 °C (15 min) 55 °C (10 min) 55 °C (3 h)

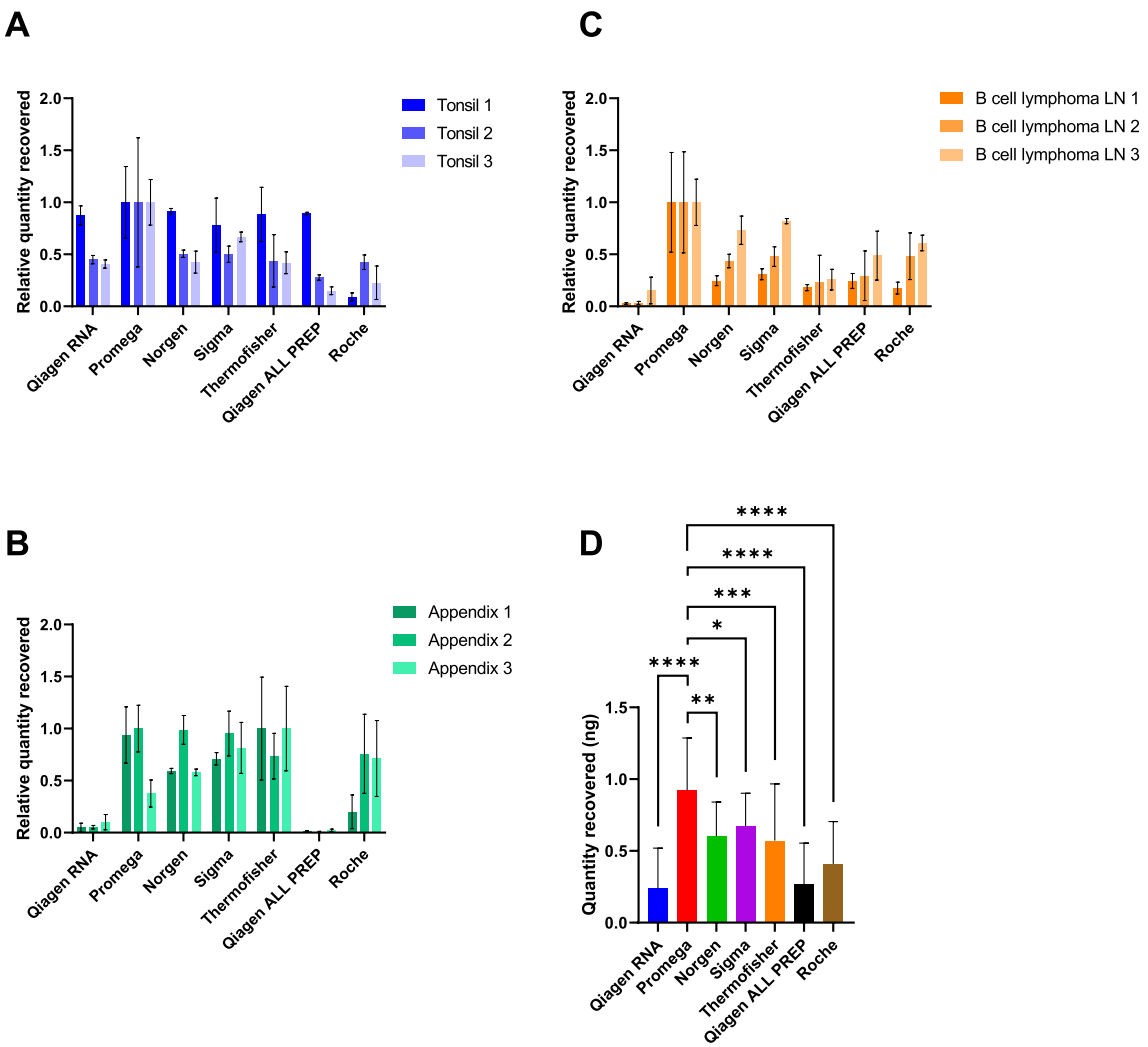

Fig. 2 Quantitative comparison of across kits and tissues. **A** Relative quantity of RNA recovered for each tonsil samples compared to best performing kit. **B** Relative quantity of RNA recovered for each appendices samples compared to best performing kit. **C** Relative quantity of RNA recovered for each B cell lymphoma lymph node samples compared to best performing kit. The average and SD of triplicates is represented. N = 3 for each bar from the bar chart for (**A**–**C**). **D** Average relative quantity recovered with each kit across the different tissue samples. N = 3 for each bar from the bar chart for (**A**–**C**) and N = 27 for each bar of (**D**). p-values were calculated with one way ANOVA Test (*p < 0.01, **p < 0.001, ***p < 0.0001, ****p < 0.00001). All p-values are provided in supplementary Table 2

(Fig. 3B). In LN (Lymph Node) samples from B-cell lymphoma, Promega and Roche kits performed significantly better than the other kits except for LN3 where Roche kit performed better than all other kits (Fig. 3C).

When considering the results of sample types altogether, Promega, Thermofisher, and Roche kits performed better in RQS quality analysis across all sample types. However, while the Thermofisher and Roche kits were statistically equivalent, the Roche kit performed slightly but significantly better than the Promega kit (as seen in Fig. 3D).

(See figure on next page.)

Fig. 3 Qualitative comparison of across kits and tissues. **A**–**C** Quality analysis by RQS of 3 tonsils (**A**), of 3 appendices (**B**) and 3 B cell lymphoma lymph nodes (**C**). **D** Average quality measured by RQS with each kit across the different tissue samples. **E**–**G** Quality analysis by DV200 of 3 tonsils (**E**), of 3 appendices (**F**) and 3 B cell lymphoma lymph nodes (**G**). **H** Average quality measured by DV200 with each kit across the different tissue samples. N = 3 for each bar from the bar chart for (**A**–**C**); **E**–**G** and N = 27 for each bar of (**D** and **H**). p-values were calculated with one way ANOVA (*p < 0.01, **p < 0.001, ***p < 0.0001, ****p < 0.00001). All p-values are provided in supplementary Table 3 and 4

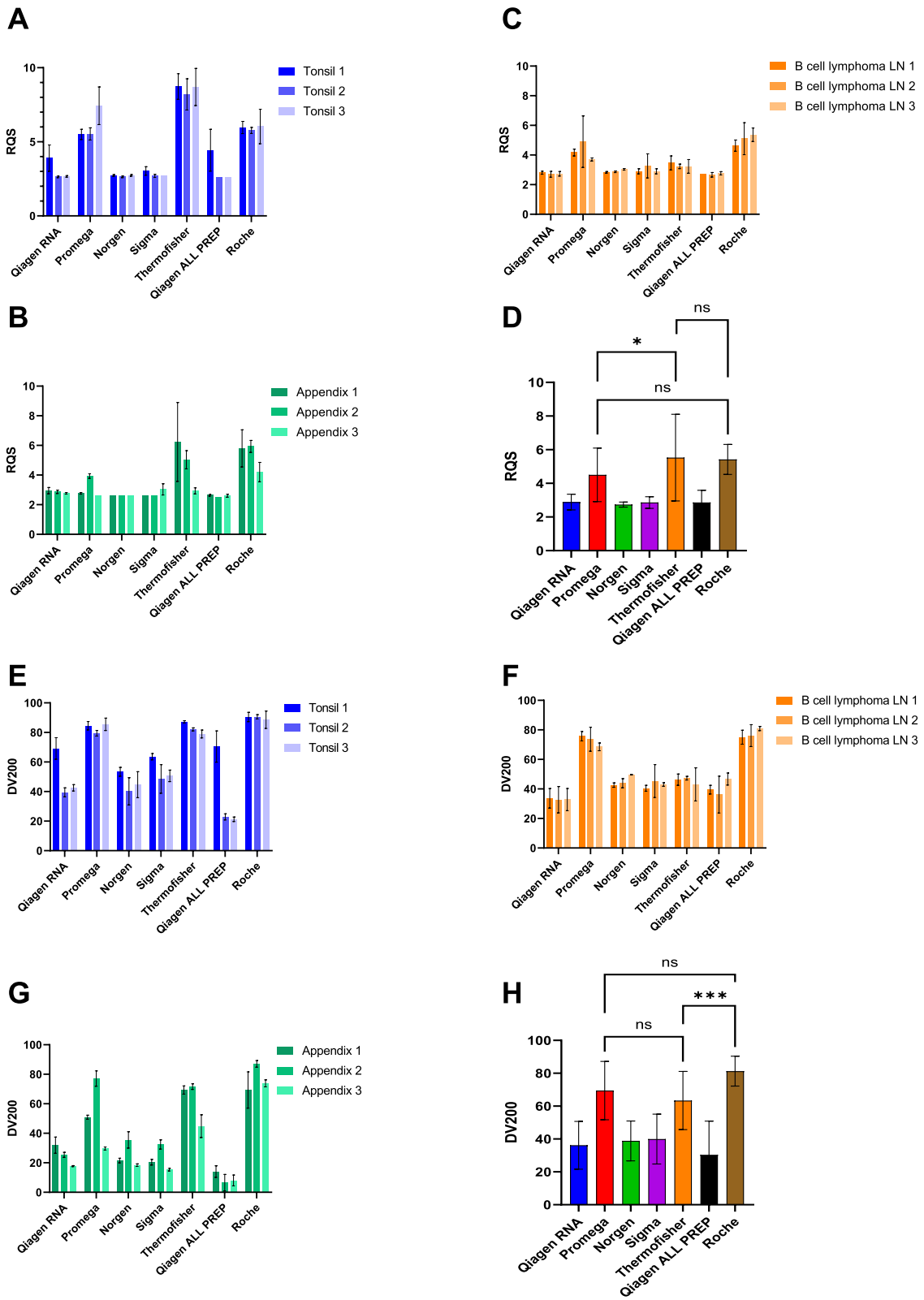

Fig. 3 (See legend on previous page.)

When analysing the DV200 values, for Tonsils 1 and 3, the kits from Promega, Thermofisher, and Roche performed similarly and better than the other kits, but in Tonsil 2, the Roche kit performed better than any other kit (Fig. 3E). For appendixes, Thermofisher and Roche kits performed better than the other kits, but the Roche kit was significantly better than any other kit for appendices 2 and 3 (Fig. 3F). Finally for samples from LN from B-cell lymphoma patients, the kits from Promega and Roche performed better than other kits, but Roche was better than any other kit in LN3 (Fig. 3G). Overall, Promega, Thermofisher, and Roche kits also performed better in DV200 quality analysis than the other kits across all sample types, but the Roche kit performed significantly better than all of them (Fig. 3H).

Multiparametric analysis

Previously, we found that the Promega kit allows for better recovery of RNA in terms of quantity than its competing kits. Kits from Promega, Thermofisher, and Roche all allow for better quality of the RNA recovered (RQS and DV200) compared to the other competing kits, with the Roche kit providing the best DV200 values across all samples. When considering RNA sequencing from FFPE samples and depending on the tissue size, some researchers may consider RQS or DV200 more important while others may choose the quantity recovered as the most critical parameter. We therefore represented each of these parameters against each other in Fig. 4. When RQS and DV200 parameters are taken together we can clearly see that of Thermofisher Promega and Roche constitute a group detached from the rest of the kits (Fig. 4A), the individual distribution of the samples shows that while samples recovered with Roche are always in the upper quadrant, some samples isolated with Promega, and even more with Thermofisher, cluster with the rest of the less performant kits (4B). It seems, therefore, that the Thermofisher kit and to a lesser extent, the kit from Promega, are more sensitive to individual variability between samples for quality performance.

When RQS or DV200 and the average quantity recovered are considered together, while on average, the kits from Promega, Thermofisher and Roche again out-perform the other kits tested here (Fig. 4C, E), only samples isolated with the Roche kit are systematically better than the other kits. The Thermofisher kit can allow exceptionally great quality recovery in some samples but largely lower quality in others. Similarly, but to a lesser extent, with the Promega kit, some samples' quality can be lower (Fig. 4D, F).

To identify the kit that offered the best compromise between quantity and quality recovered, we created a score by multiplying the relative quantity recovered by DV200 (offering the best dynamic range of analysis between DV200 and RQS). Using this score, we show that Promega significantly outperforms all other kits (Fig. 4G), despite presenting a lower score than other kits in some particular samples (Fig. 4H).

Discussion

When extracting RNA from FFPE samples for RNA sequencing or RT-PCR, multiple parameters must be taken into consideration, including the quantity and quality of RNA recovered to allow proper sequencing and coverage for correct data analysis. In the study presented, some kits were systematically outperformed by others, both in terms of quality and quantity recovered. Among them, the historically commonly used kit from Qiagen (both RNA-specific and ALL PREP) was a surprise to us.

In terms of quantity recovered, the Promega kit performed better on average than any of the other kits, but this could have come at the expense of quality of the recovered RNA in some samples. Therefore, in cases of small biopsies or small samples, this kit might be the best choice to maximize the recovery rate. However, it is important to note that in some cases, we observed large standard deviations in the quantity recovered between triplicates of the same sample (including with Promega). Our hypothesis is that in some cases, the column used can be slightly clogged, resulting in less volume recovered after final centrifugation and therefore, higher concentration. This would not affect the quality of the RNA recovered, explaining why less variation was observed in terms of RQS and DV200.

On the other hand, if sample size is not an issue, and the choice is made to maximize the quality of the sample,

(See figure on next page.)

Fig. 4 Multiparametric analysis. **A**, **B** Comparison of quality analysed by RQS and DV200. **A** Representation of all samples tested. **B** Average and SD of RQS and DV200 across the different tissue samples. **C**, **D** Comparison of quality analysed by RQS and average relative quantity recovered with each kit across the different tissue samples. **C** Representation of all samples tested. **D** Average and SD of RQS and average quantity across the different tissue samples. **E**, **F** Comparison of quality analysed by DV200 and average relative quantity recovered with each kit across the different tissue samples. **E** Representation of all samples tested. **F** Average and SD of DV200 and relative quantity across the different tissue samples. **G** Comparison of score including quality and quantity by multiplying relative quantity recovered by DV200 for each sample. N = 27 for each bar of (**G**) ($p < 0.0002$ between Promega and Thermo kits, $p < 0.0001$ between Promega and all other kits). **H** Box plot of relative quantity x DV200 score for each sample. All adjusted p-values are provided in supplementary Table 5

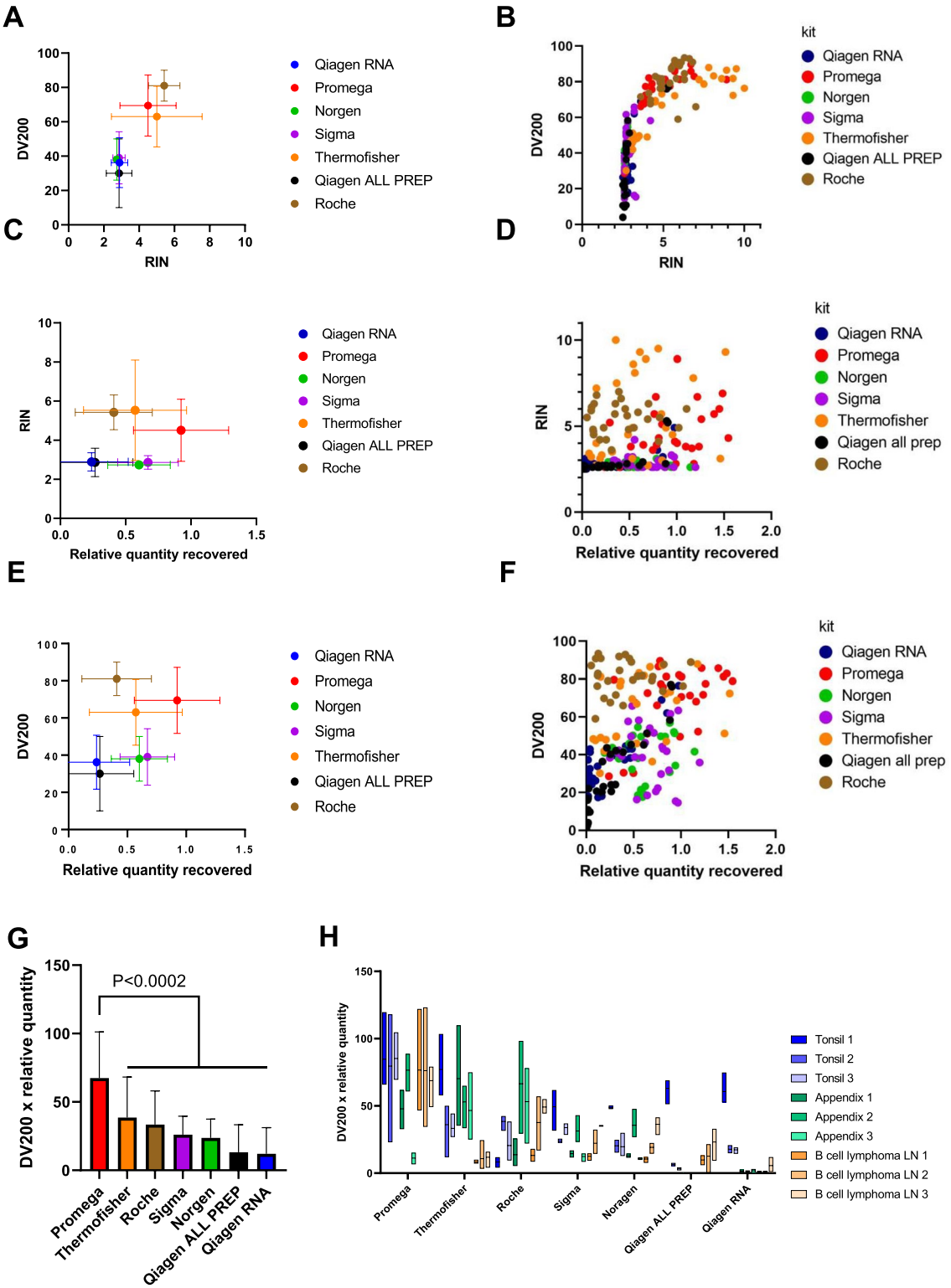

Fig. 4 (See legend on previous page.)

one might consider using the Roche kit, which provided a nearly systematic better-quality recovery than other kits.

When trying to identify the best compromise between quantity recovered and quality recovered, a score was created, taking into account both quantity recovered and quality (as measured by DV200). In that analysis, it was observed that Promega gave a significantly better compromise than other kits.

It is very important to notice that while we chose to use the minimum elution volume recommended for each kit, increasing the elution volume often can result in increased recovery of RNA with lower concentration.

In our study, we used relatively recent samples, less than one year old. However, Ahlfen et al. reported that older FFPE samples typically exhibit poorer RNA quality compared to newer ones [14]. Testing these kits with samples of various ages, including decades-old specimens, could be valuable to evaluate recovery efficiency on more degraded samples.

In any case, the quality and quantity of RNA recovered can vary greatly between tissue types and samples, likely due to factors such as protein content, fixation parameters, or cell types present within the sample. The standard practice of tissue fixation can vary considerably between institutions and laboratories, and it is important to evaluate the impact of fixation time on the quality and quantity recovered with various kits. Additionally, the thickness of the section used can also affect the results. Some studies have shown that sections with a thickness of 10 µm or more perform better than finer sections, but too thick sections > 40um might give opposite results [15, 16]. In each laboratory specific settings, some kits that have been shown to perform not so well in this study, have perform better than their competitors. To get the best results for a specific type of tissue and sample, it is advisable to test a variety of kits. Indeed, when comparing our results to the literature, our analysis was conducted on fewer samples than that of Gouveia et al. [17], but we evaluated more commercially available kits. There are also other kits available on the market, and our study does not represent an exhaustive list of the possibilities offered to researchers and clinicians. Other studies have identified alternative kits that performed better with their tested samples. For instance, Boeckx et al. found that Qiagen kits outperformed the alternatives they tested [18] although none of those alternative kits were included in our analysis. Similarly, Hafezi et al. demonstrated that the Qiagen RNeasy FFPE Kit showed superior performance compared to other tested alternatives (not included in our study) [19]. Finally, Patel et al. concluded that the Qiagen AllPrep kit outperformed the kits they tested, including the PureLink FFPE RNA Isolation Kit (Thermo) and the High Pure FFPET RNA Isolation Kit (Roche), which we also tested and found to perform better than the Qiagen AllPrep kit [20].

In our experimental setup, we did not perform RT-PCR or RNA sequencing to validate the quality of the recovered RNA. Having performed the nucleic acid analysis of all the samples at the same time, we believe that this ensures a reliability of our comparison. Also, for us and many other institutions nucleic acid analysis serves as an excellent preliminary assessment tool. It ensures that only high-quality RNA samples are selected for more detailed and expensive downstream applications, such as sequencing. Nucleic acid analysis is a robust and efficient method for evaluating RNA quality and quantity. It provides comprehensive data on RNA integrity and concentration, making it sufficient for the initial assessment of RNA samples.

Although some reports, such as Marczyk et al. [21], have documented that the choice of RNA purification kit from FFPE samples does not significantly influence the overall quality of results from whole transcriptome RNA sequencing, it is important to note that while RNA can be recovered in acceptable quantity and quality (assessed by RQS and DV200), there can still be residual contaminants and formalin adducts that could inhibit PCR and reverse transcription.

Typically, to remove formalin adducts, heat is applied during the extraction protocol, but this also causes RNA fragmentation. All the kits we tested use heat application in that regard. Therefore, kits that apply less heat might yield RNA that appears more intact in electrophoresis assays (RQS and DV200), but this RNA may be less suitable for RT-PCR and PCR (and consequently sequencing) due to less effective removal of formalin adducts. When comparing the recommended heat strategies across the different kits tested here, we found that the Promega procedure, which provided us with the best combination of RNA quantity and quality, also used the longest and highest heat application, thereby reducing the potential risk of residual formalin adducts the most. Despite reports such as Marczyk et al. [21] suggesting that kit selection does not influence RNA-sequencing quality, it is also reported that with modifications in temperature and incubation time, some kits could perform better on specific tissue types [19], stressing once more the need of testing and optimalisation for each tissue type and each institution.

All kits tested in this study use nuclease-free water for RNA recovery; however, extraction procedures and cleaning steps may impact any residual contaminants in the final RNA eluate. We did not evaluate the long-term impact of these residues on RNA storage stability, but it is recommended to use the extracted RNA shortly after extraction and avoid extended storage. The value of using FFPE samples lies in their extended room-temperature

storage capacity, minimizing RNA degradation concerns typically associated with fresh samples [22].

When conducting large-scale extractions or routine isolations in pathology laboratories, the per-sample cost may become a significant consideration. While the cost difference per sample (reported in Table 1) may seem minor for small-scale analyses (ranging from $7.9 for the least expensive to $16.12 for the costliest), it can be impactful in larger-scale operations. It is challenging to directly compare the cost of the Qiagen AllPrep kit, as it extracts both DNA and RNA, whereas other kits would require an additional purchase to obtain DNA. The prices shown are based on our institution's rates, which may vary by location. Notably, the Promega kit, which provided superior results in both RNA quality and quantity, but not the cheapest, priced at approximately 12% more expensive than the cost of the Thermo Fisher kit. The latter yielded comparable DV200 quality and a slightly higher RQS score than the Promega kit. Therefore, institutions may wish to evaluate their own pricing and consider whether quality and cost should take precedence over quantity, particularly when large sample sizes are available. While these manual protocols require similar hands-on times, and thus similar personnel costs, the wide range of incubation times (shown in Tables 1 and 2) could be a factor to consider. Longer incubation times may provide flexibility for multitasking, whereas shorter incubation times may require closer attention.

Finally, some companies offer automated systems that employ similar procedure as the one tested in manual kits here. Automation can be particularly useful when analysing larger cohorts or ensuring better reproducibility. Automated systems streamline the RNA extraction process, reducing human error and variability, which is crucial for consistency across numerous samples. This standardization leads to more reproducible results and higher throughput, allowing researchers to process more samples in less time. Finally, cost considerations related to the purchase of instruments and associated kits may also be relevant when planning routine or large-scale operations. Automated systems also minimize hands-on time, freeing up laboratory personnel for other tasks, and often include advanced software for real-time monitoring and quality control, ensuring the integrity of the RNA extraction process.

Conclusion

In conclusion, this study highlights the critical importance of selecting the appropriate RNA extraction kit when working with FFPE samples, as variations in both quantity and quality of RNA recovered can significantly impact downstream applications. Among the kits tested here, the Promega kit showed superior performance in terms of RNA quantity and quality recovery, making it a suitable choice for small samples, though quality variations were noted. Conversely, the Roche kit consistently provided higher-quality RNA, particularly in cases where sample size was not a limiting factor. The study also underscores the need for careful consideration of tissue type and fixation parameters, as these factors can influence RNA recovery. The results emphasize that no single kit is universally superior; instead, optimization and testing for specific tissue types and laboratory conditions are essential for achieving the best outcomes.

Abbreviations

RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
RQS	RNA quality score
DV200	Percentage of RNA fragments > 200 nucleotides
FFPE	Formalin-Fixed Paraffin-Embedded
RT-PCR	Real-time polymerase chain reaction
RNAseq	RNA sequencing
LCM	Laser capture microdissection
H&E stain	Hematoxylin and eosin stain
LN	Lymph node

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-024-05890-5.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

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Author contributions

W.M. selected all the samples, S.D. cut all the samples, S.A., S.D., C.R., A.S. performed the experiments, L.L., S.T. performed the RQS and DV200 analysis, C.R., D.B., performed the analysis C.R., drafted the manuscript and the figures, W.H., S.H., E.A., S.S. and A.J. edited the text. Conceptualization by W.M., C.R., D.B. and W.H., project supervision and coordination C.R. All authors reviewed the manuscript.

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Availability of data and materials

The datasets generated and/or analysed during the current study are available in the supplementary Table 1.

Declarations

Ethics approval and consent to participate

Lymph nodes from patients affected by B-cell lymphoma were anonymised and used under IRB #150900 approved by Sidra IRB committee in accordance with Qatari Regulations, Sidra Medicine policies and procedures and all applicable regulations related to human research protection. Patients were not

consented as sample were anonymised, therefore human Ethics and Consent to Participate declarations is not applicable. Tonsil and Appendix samples were anonymized and considered as surgical waste and not subjected to IRB approval.

Consent for publication
"Not applicable".

Competing interests
The authors of this manuscript have no financial or intellectual conflict of interest to disclose.

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