UDC 664.9; 639.21 https://doi.org/10.15407/biotech16.01.067

LOSS OF AN ABUNDANT QUANTITY OF RIBONUCLEIC ACID DURING MINI COLUMN ISOLATION METHOD

Sudhir Bhatia Genekam Biotechnology AG, Duisburg, Germany

E-mail: anfrage@genekam.de

Received 2022/04/28
Revised 2023/06/01
Accepted 2023/06/30

Aim. The Isolation of nucleic acid is an important step for conducting different molecular assays in many laboratories around the world. It is also a common practice that user is isolating the ribonucleic acid (RNA) from the samples with mini column once and throwing away the supernatant. This makes isolated RNA as limiting factor in many studies as this issue has not been addressed in literature. Therefore, we decided to conduct whether it is a loss of ribonucleic acid during the mini column isolation method.

Method. In this research, we have made analysis whether there is a loss of RNA during the isolation process. The mini column isolations were done with different samples of human tissues from placenta and umbilical cords. These were assessed with spectrometric instrument and real time PCR machine.

Results. It was found that there is loss of abundant quantity of RNA during the subsequent isolations. The amount measured with UV spectrometer indicates that some times 2nd and 3rd isolation have more RNA than the first isolation. Realtime PCR for house keeping gene beta actin shows that presence of RNA can be seen up to 6 isolation cycles from supernatants.

Conclusion. There is loss of RNA in subsequent isolations with mini column method, therefore it is possible to isolate more RNA from the next supernatant isolations. User should do the multiple isolations to get higher yield of RNA.

Key words: mini column isolation, RNA; nucleic acid; ribonucleic acid; polymerase chain reaction; real time PCR; viruses; biomarkers; vaccines.

Isolation of nucleic acid is an essential step for conducting the different nucleic acid analysis methods like PCR and hybridization for various viral, bacterial, biomarkers and genetic targets [1–4]. Not only this, the nucleic acid can be used as therapeutic as well as vaccine options in many clinical settings, therefore there is an urgent need to isolate the sufficient amount of nucleic acid in highly pure form [5–8]. Ribonucleic acid (RNA) can be isolated from different sources e.g., blood, tissue, buccal swabs, nasal samples, plasma, serum, urine and cell cultures. There are different methods available to isolate the RNA, which are magnetic beads, solutions-based and mini column methods for examples. The mini column isolation method is widely used in the laboratories througaround the world. Usually most of the laboratories isolate the RNA from the sample once and the supernatant of this method is discarded. Many times, there is limited volume of isolated RNA e.g., 50 or 100 µl available, which is not sufficient to conduct the whole studies, where user has to conduct a set of experiments on the same isolated RNA (homogenous material). In case of development of vaccines, one needs sufficient quantity of RNA, which may not be available from a single isolation source [4, 9, 10].

In this study, the experiments are conducted to find whether RNA is still in supernatant and this surplus RNA can be isolated to conduct the further analysis.

A protocol of local ethics committee approval of Genekam Biotechnology AG (Number: GEN01-23) about conducting the experiments of this research work is available. In this experiment, 4 samples from different tissues like umbilical cord, placenta tissue, placenta membrane etc. are used to isolate the RNA with mini column RNA isolation kit

(Genekam, Germany). 100 µl of the samples were added to lysis buffer with proteinase K. These samples were kept at 56 °C for 10 min. After that guanidine solution was added, while keeping at 70 °C for 5 min. 400 ul of molecular ethanol (Applichem, Germany) was added to each well.

Mini column step. These mixtures were added to mini columns, which were centrifuged at the speed of 11000 rpm for 1 minutes. The supernatants from each sample were collected in separate tubes. Mini columns were washed with 500 µl B and C tubes each while centrifuging at the speed of 11 000 rpm for one minute. At the end mini columns were centrifuged in order to make them dry at the speed of 13 000 rpm for 1 minute. At last, 70 °C warm elution buffer was added to each mini column, while keeping this for 2 minutes at room temperature. In order to get the elution buffer containing RNA, the mini columns were centrifuged. The tubes were labelled as first isolation and stored at 4 °C for further analysis.

Collected supernatants were processed as mentioned in mini column step till there was use of elution buffer at the end to elute RNA. During this step, the supernatants were collected and processed again as mentioned above. There were two groups. In one group, there were 2 isolations (one original isolation and 1 isolation from supernatants, these are labelled as Isolation 1 and 2) and in 2nd group, there are 7 isolations (one original isolation and 6 subsequent isolations from supernatants, which were labelled as Isolation 1 to 7). In both groups, isolation 1 is the original and first isolation and rest isolations are from subsequent supernatants. (Tables 1 and 2).

Total Volume of isolated RNA was 50 µl per isolation. The full RNA isolation protocol can be also obtained from manufacturer.

Measurement of RNA content with UV spectrometer, Nanodrop (Thermofischer, USA): The instrument was calibrated with elution buffer. After that, 2 ul of each isolated was measured and the values are recorded at µg/2 µl.

Real time PCR for human internal control. All isolated samples were tested for the presence of RNA with housekeeping gene (β-Actin) through real time kit FR799 (Genekam, Germany) in 96 well plate in a real time machine ABI 7500 (Thermofischer, USA). 2 ul of isolated RNA was used during the real time PCR reaction in 18 ul RNA mastermix containing cDNA synthesizing enzymes. The temperatures and cycling conditions were one cycle 42 °C for 60 minutes and 70 °C for 10 min as well as 45 cycles of 90 °C for 15 s and 60 seconds for 60 °C. The results were analyzed with software in linear and log modes along with recording of Ct values. The full protocol for conducting real time PCR for internal control can be obtained from the manufacturer.

Group 1, where the 2 isolations were done. The values of these versions of different samples measured with spectrometer is shown in the Table 1. The values indicate that there is loss of RNA during 2 isolations. Therefore, it is decided to conduct second experiment, where the isolation was repeated up to 7 isolations. The results are shown in the Table 2. The results indicate that there is a loss of RNA during all steps and this loss decreases with the increase in the number of isolations. In the sample 2, it varies better 0.170 µg/2 µl at the beginning to 0.006 µg / 2 µl. It was very surprising to see that yield of isolated RNA was more in the first supernatant passage (second isolation) than the first isolation.

Real time PCR of isolated RNA in different isolated versions show that real time for beta actin housekeeping gene is in position to detect RNA in all isolations regardless whatever quantity was detected in spectrometer. Results are shown as curves in the Figure. It is hard to find any correlation between the amount of RNA measured in spectrometer and Ct values achieved in the real time machine.

Since the availability of possibility to isolate the nucleic acid with mini columns, it has become a common practice in all laboratories working with molecular methods, to conduct the RNA isolation from different samples to analyze them for different targets as these may be pathogenic agents and biomarkers of different cells e.g., stem cells, tumor cells, immune cells etc. It is also daily

Table 1

Measurement of UV spectrometer for the concentrations of RNA in different isolations (Group 1)

Sample *	Isolation 1	Isolation 2
Sample 1	0.006	0.009
Sample 2	0.053	0.042
Sample 3	0.005	0.022
Sample 4	0.005	0.09

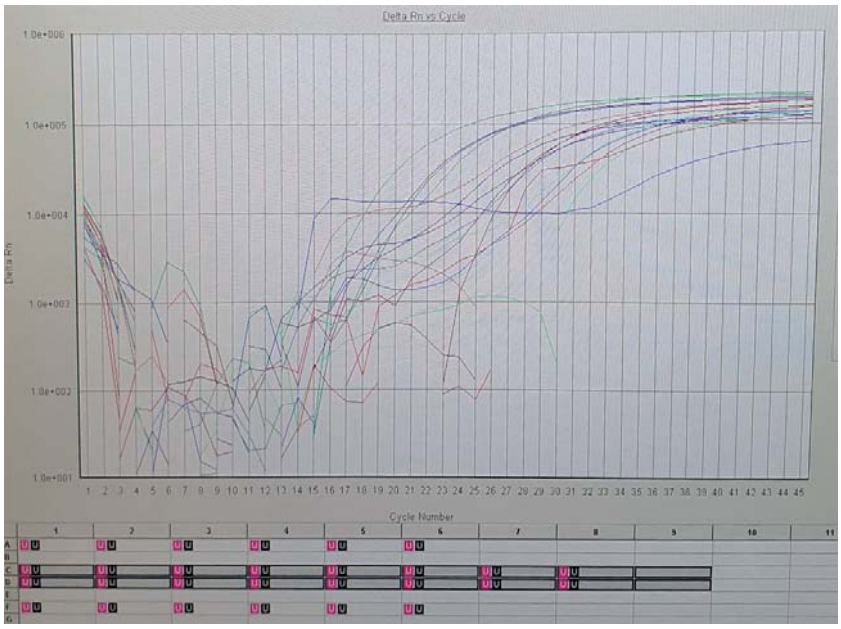
* µg / 2µl.

Table 2

Measurement of UV spectrometer for the concentrations of RNA in different isolations (Group 2)

Sample *	Isolation 1	Isolation 2	Isolation 3	Isolation 4	Isolation 5	Isolation 6	Isolation 7
Sample 1	0.005	0.003	0.002	0.002	0.002	0.008	0.001
Sample 2	0.018	0.007	0.004	0.001	0.001	0.008	0.006
Sample 3	0.077	0.163	0.073	0.001	0.001	0.001	0.003
Sample 4	0.01	0.4	0.136	0.014	0.002	0.002	0.005

* µg / 2µl.



The results of real time PCR for internal control for the presence of human RNA in isolated samples

practice in the laboratories to measure the presence of viral RNA in different samples. [1, 2, 11–13] Most of the laboratories are doing only once the isolation and throw away the samples as supernatant. Amount of isolated RNA with only one-time isolation is very limited e.g., 100 µl. If user is conducting experiments for a number of different targets, this small amount can be a limiting factor because of its small size.

In the literature, there is hardly to find any report that some groups have tried to look into this important problem in spite of the fact that each group needs sufficient amount of nucleic acid to conduct the assays from the same source. This study is indicating that it is possible to isolate the more RNA from the supernatants of the same sample. In this way, many laboratories have sufficient amount of isolated RNA to conduct bigger studies and compare them also [14].

There are research groups, which are working on development of vaccines and therapies, they may face many times a challenge to isolate the sufficient RNA to conduct their experimental studies. This publication provides them the way to isolate more amount of valuable RNA rather throwing of the rest of supernatant.

These studies shown here can help many institutes and laboratories to save money in terms of chemicals and time used to isolate the samples. In two samples shown Table 2, the amounts of isolated RNA from sample number 3 and 4 were less than that of RNA isolated from two following supernatants from the first isolation. It should be investigated further as it indicates that there may be some time a lot of loss of RNA in the first isolation or binding capacity of membrane may be playing a role. User can have more isolated RNA in 2nd and 3rd subsequent isolations.

There are many publications, where research groups are using 5 or 10 µl during the PCR studies, hence a lot of quantity of isolated RNA can be obtained from supernatant, which is usually thrown away as waste product. [15, 16] This publication is showing a way to get larger volume of isolated RNA to bigger studies.

Fundin. This work is self-funded and there is no external funding.

Acknowledgment. The author is thankful for assisting this work to Ms Kornelia Niklis in laboratory work and Mr Stephan Suppers for graphic design.

REFERENCES

1. *Lee K. O., Park M. Y., Kim L. H., Seong H. S., Park B. H., Jeong S. J.* Pandemic Novel Influenza A (H1N1) Virus in Korea: The Experience from August to September 2009. *Korean J Clin Lab Sci*. 2009, 41, 145–152.
2. *Lee S. H., Park B. H., Kang H. Y., Lee K. O., Kim G. Y.* Interim report on pandemic H1N1 influenza virus infections in Korea, August to October, 2009. *Abstracts 20th European Congress of Clinical Microbiology and Infectious Diseases; European Congress of Clinical Microbiology and Infectious Diseases; Vienna, Austria*, 10–13 April, 2010.
3. *Pruvot M., Kamyingkird K., Desquesnes M., Sarataphan N., Jittapalapong S.* The effect of the DNA preparation method on the sensitivity of PCR for the detection of Trypanosoma evansi in rodents and implications for epidemiological surveillance efforts. *Vet Parasitol*. 2013, 191(3–4), 203–208. https://doi.org/10.1016/j.vetpar.2012.09.010
4. *Bhatia, Sudhir.* First suspected case: detection of an unknown pathogenic human coronavirus in post vaccination case. *figshare. Preprint*. 2021. https://doi.org/10.6084/m9.figshare.17014487.v1
5. *Wolff J. A., Malone R. W., Williams P., Chong W., Acsadi G., Jani A., Felgner P. L.* Direct gene transfer into mouse muscle *in vivo*. *Science*. 1990, 247(4949), 1465–1468. https://doi.org/10.1126/science.1690918
6. *Schlake T., Thess A., Fotin-Mleczek M., Kallen K. J.* Developing mRNA-vaccine technologies. *RNA Biol*. 2012, 9(11), 1319–1330. https://doi.org/10.4161/rna.22269
7. *Phua K. K. L., Leong K. W, Nair S. K.* Transfection efficiency and transgene expression kinetics of mRNA delivered in naked and nanoparticle format. *JCR*. 2013, 166 (3), 227–233. https://doi.org/10.1016/j.jconrel.2012.12.029
8. *Wadhwa A., Aljabbari A., Lokras A., Foged C., Thakur A.* Opportunities and Challenges in the Delivery of mRNA-Based Vaccines. Pharmaceutics. 2020, 12, 102. https://doi.org/10.3390/pharmaceutics12020102
9. *Boom R., Sol C. J., Salimans M. M., Jansen C. L., Wertheim-van Dillen P. M., van der* Noordaa J. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol*. 1990, 28(3), 495–503. https://doi.org/10.1128/jcm.28.3.495-503.1990
10. *Zhang W., Wu D., Wei J., Xoao G.* A new method for the detection of the H5 influenza virus by magnetic beads capturing quantum dot fluorescent signals. *Biotechnol Lett*. 2010, 32, 1933–1937. https://doi.org/10.1007/s10529-010-0379-5
11. *Silva A. I., Tomé M. J., Correia A. E., Passos-Coelho J. L.* Human mammaglobin RT-PCR assay for detection of occult breast cancer cells in hematopoietic products. Ann Oncol. 2002, 13 (3), 422–429. https://doi.org/10.1093/annonc/mdf107
12. *Chechlińska M., Kowalewska M., Markowicz, S., Nowak R.* The relevance of RT–PCR markers for metastatic tumour cell detection. *Br J Cancer*. 2006, 94, 1761. https://doi.org/10.1038/sj.bjc.6603131
13. *Othman A. A., Eissa A. A., Markous R. D., Ahmed B. D., Al-Allawi N. A.* Hepatitis C virus genotypes among multiply transfused hemoglobinopathy patients from Northern Iraq. Asian *J Transfus Sci*. 2014, 8(1), 32–34. https://doi.org/10.4103/0973-6247.126687
14. *Hønsvall B. K., Robertson L. J.* Washed Away; How Not to Lose Your RNA during Isolation. *J Biomol Tech*. 2017, 28(2), 75–79. https://doi.org/10.7171/jbt.17-2802-004
15. *Moës E., Vijgen L., Keyaerts E., Zlateva K., Li S., Maes P., Pyrc K., Berkhout B., van der Hoek L, Van Ranst M.* A novel pancoronavirus RT-PCR assay: frequent detection of human coronavirus NL63 in children hospitalized with respiratory tract infections in Belgium. *BMC Infect Dis*. 2005. 1, 5–6. https://doi.org/10.1186/1471-2334-5-6
16. *Holbrook M. G., Anthony S. J., Navarrete-Macias I., Bestebroer T., Munster V. J., van Doremalen N.* Updated and Validated Pan-Coronavirus PCR Assay to Detect All Coronavirus Genera. Viruses. 2021, 13(4), 599. https://doi.org/10.3390/v13040599

ВТРАТА ЗНАЧНОЇ КІЛЬКОСТІ РИБОНУКЛЕЇНОВОЇ КИСЛОТИ ЗА УМОВ ЇЇ ВИДІЛЕННЯ З ВИКОРИСТАННЯМ МІНІ-КОЛОНКИ

Sudhir Bhatia

Genekam Biotechnology AG, Дуйсбург, Германія

E-mail: *anfrage@genekam.de*

Мета. Виділення нуклеїнової кислоти є важливим кроком для проведення різних молекулярних аналізів у багатьох лабораторіях по всьому світу. Також поширеною практикою є те, що користувач один раз виділяє рибонуклеїнову кислоту (РНК) із зразків за допомогою міні-колонки та викидає супернатант. Це робить ізольовану РНК обмежувальним фактором у багатьох дослідженнях, оскільки це питання не розглядалося в літературі. Тому ми вирішили визначити, чи є це втратою рибонуклеїнової кислоти під час методу ізоляції на мініколонці.

Метод. Проводили виділення в міні-колонці з різними зразками людських тканин із плаценти та пуповини та подальші виділення супернатантів. Виходи та успішні виділення РНК оцінювали за допомогою спектрометричного приладу та машини ПЛР у реальному часі.

Результати. Було виявлено, що при наступних виділеннях відбувається втрата великої кількості РНК. Кількість, виміряна за допомогою УФ-спектрометра, вказує на те, що іноді 2-й і 3-й виділення містять більше РНК, ніж перше виділення. ПЛР у реальному часі для бета-актину гена домашнього обслуговування показує, що присутність РНК можна побачити до 6 циклів ізоляції з супернатантів.

Висновок. При наступних виділеннях за допомогою методу міні-колонки відбувається втрата РНК, тому можна виділити більше РНК із наступних виділень супернатанту. Користувач повинен виконати багаторазові виділення, щоб отримати більший вихід РНК.

Ключові слова: виділення мініколонки, РНК; нуклеїнова кислота; полімеразна ланцюгова реакція; ПЛР в реальному часі; віруси; біомаркери; вакцини.