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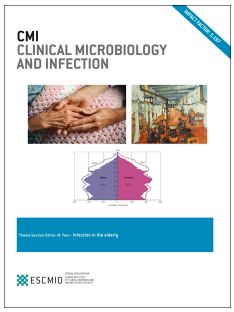

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PII: S1198-743X(21)00324-4

DOI: https://doi.org/10.1016/j.cmi.2021.06.007

Reference: CMI 2571

To appear in: *Clinical Microbiology and Infection*

Received Date: 25 March 2021

Revised Date: 25 May 2021

Accepted Date: 5 June 2021

Please cite this article as: Grau S, Ferrández O, Martín-García E, Maldonado R, Reconstituted mRNA Covid-19 vaccines may maintain stability after continuous movement, *Clinical Microbiology and Infection*, https://doi.org/10.1016/j.cmi.2021.06.007.

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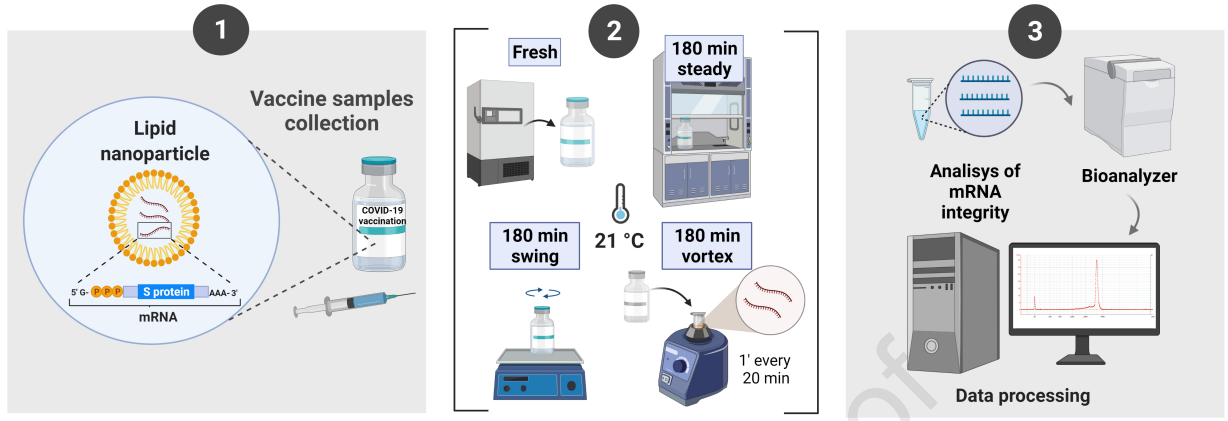

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Reconstituted mRNA Covid-19 vaccines may maintain stability after continuous movement

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Abstract

Objectives: There is an urgent need to ameliorate the possibilities to transport the reconstituted mRNA vaccines from the centralized preparation centers to the vaccination sites to improve the COVID-19 vaccination campaigns. We have analyzed Pfizer-BioNTech and Moderna vaccine integrity under different movement conditions to provide information that may improve the distribution of vaccines to the target population.

Methods: Syringes of reconstituted Pfizer-BioNTech or Moderna COVID-19 vaccines were prepared in a laminar flow chamber to be subjected to a stability analysis in order to evaluate the impact of movement conditions on mRNA integrity. RNA integrity was checked by the lack of RNA peaks under the original mRNA peak in the electropherogram resulting from potential fragments from RNA degradation. Samples were then exposed for 180 min at room temperature (21±1C°, 55±10% of humidity) under different movement conditions.

Results: We report that the integrity of the mRNA of the COVID-19 reconstituted vaccines after continuous moderate movement at room temperature is maintained for at least three hours with values of fluorescence units (FU) under the original mRNA peak of 0.38±0.06 in Pfizer-BioNTech and 0.96±1.18 FU in Moderna, equal to the values obtained without movement (0.36±0.08 FU in Pfizer-BioNTech and 1.12±0.19 FU in Moderna). In contrast, the integrity of these vaccines exposed to repeated Vortex shaking was significantly impaired ($p<0.001$) with values under the original mRNA peak of 1.34±0.31 FU for the Pfizer-BioNTech and 5.03±1.16 FU for the Moderna samples.

Conclusions: The stability of these reconstituted vaccines here reported may improve the efficiency in the ground transportation and distribution of the vaccines, which may lead to shorter and more homogeneous vaccination in cities and rural areas.

Keywords: Vaccination, Covid-19, Transportation, SARS-CoV2.

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Introduction

Pfizer-BioNTech and Moderna vaccines are composed of mRNA, and the main difference lies in the lipid nanoparticles that integrate the vaccines to protect the mRNA integrity and facilitate cell entry. Due to this difference, the Pfizer-BioNTech vaccine requires storage in freezers between -90ºC to -60ºC, whereas the Moderna vaccine should be stored between -25ºC to -15ºC [1]. Pfizer-BioNTech vaccine reception in hospitals entails their reconstitution and re-storage at temperatures between 2ºC-8ºC with an expiration of 5 days, while the expiration is only 2h at room temperature. Moderna vaccine can be stored refrigerated between 2ºC-8ºC for 30 days and between 8ºC-25ºC for up to 12h before use. When the first dose is withdrawn from the vial, both vaccines should be held between 2ºC-25ºC and discarded 6h later.

It is recommended that reconstituted vials of the Pfizer-BioNTech and Moderna vaccine should not be transported to avoid unnecessary movement that could alter mRNA integrity, which represents a major limitation for the rapid spread of vaccination [1,2]. However, no information is currently available about the consequences of movement in the reconstituted vaccines' integrity. We have now analyzed Pfizer-BioNTech and Moderna vaccine integrity under different movement conditions and provide novel information crucial to improve the vaccines' distribution to the target population.

Materials and Methods

The Hospital del Mar (Barcelona, Spain) acted as a reference hospital and the Pharmacy department as a vaccine distributor. Several vials and syringes of Pfizer-BioNTech COVID-19 and Moderna vaccines were returned to the pharmacy department by the vaccination teams after exceeding the expiration time or potential microbiological contamination, such as vials falling to the ground. We selected for this analysis only the vials and syringes with loss of guarantee of lack of microbiological contamination. Syringes of reconstituted Pfizer-BioNTech or Moderna COVID-19 vaccines (0.5-2 ml) were prepared in a laminar flow chamber to be subjected to a stability analysis to evaluate the impact of movement conditions on mRNA integrity.

For this purpose, samples were exposed for 180 min at room temperature (21±1C°, 55±10% of humidity) under different conditions. A first group (n=12 from Pfizer-BioNTech and n=17 from Moderna) remained without movement during 180 min to mimic the manufacturer's vaccine conditions use after reconstitution. A second group (n=10 from Pfizer-BioNTech and n=16 from Moderna) was exposed to continuous movement during 180 min in swing shaker (Ovan SW-3DE) at 40rpm with an inclination angle (± 8°) to mimic the movement that may occur in ground transportation under the most unfavorable road conditions. A third group (n=8 from Pfizer-BioNTech and n=14 from Moderna) was exposed to 1 min Vortex (Scientific Industries SI™ Vortex-Genie™ 2) at 3200rpm every 20 min during 180 min, a massive shaking reported to impair RNA integrity [3]. A small amount (2µL) of the fresh samples (n=12 from Pfizer-BioNTech and n=23 from Moderna) was extracted before this period under sterile conditions and immediately analyzed to also compare the integrity of the samples before and after exposure to these conditions.

Microfluidic measurements to analyze mRNA integrity were performed using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) with the RNA 6000 Nano LabChip kit and the assay Eukaryote total RNA Nano (Genomics Core Facility, University Pompeu Fabra). Results were generated and analyzed with the Bioanalyzer 2100 Expert Software (Version B.0210.SI764) and manual integration combined with smear analysis was used to define regions following the Bioanalyzer user guide. This technique has been validated for evaluation of RNA integrity with high accuracy and precision [4], and it is widely used to determine such RNA integrity [5].

One-way ANOVA (group as between-subjects factor) was used followed by multiple-group comparisons (Newman Keuls) when the main variable was significant ($p<0.05$) using the Statistical Package for Social Science program SPSS 20.0. Results are expressed as mean ± SEM. The study achieved a power of 99% with our sample size of 8-23 per group.

Results

Our results revealed a negligible mRNA degradation that was non-significant in the Pfizer-BioNTech and Moderna samples exposed to room temperature for 180 min with

(Pfizer-BioNTech: 0.38±0.06 fluorescence units, FU; Moderna 0.96±1.18FU) or without swing shaker (Pfizer-BioNTech: 0.36±0.08FU; Moderna: 1.12±0.19FU) when compared to the fresh samples (Pfizer-BioNTech: 0.26±0.07FU; Moderna: 0.91±0.16FU). Indeed, the RNA fractions areas under the original mRNA peak were similar in these three experimental groups with very low FU values (Figure 1a and 2a) that correspond to less than 5% of original mRNA degradation for both Pfizer-BioNTech and Moderna samples (Figure 1b and 2b). Any possible product of the original mRNA's degradation should be identified in these lower molecular weight fractions [4].

In contrast, mRNA integrity of Pfizer-BioNTech and Moderna samples exposed to Vortex shaking was significantly impaired. Indeed, RNA fractions area under the original mRNA peak was 1.34±0.31FU (P<0.001) for the Pfizer-BioNTech samples (Figure 1a): 9.87±2.36% of original mRNA degradation (P<0.01) (Figure 1b). RNA fractions area under the original mRNA peak of the Moderna samples after Vortex was 5.03±1.16FU (P<0.001) (Figure 2a), corresponding to only 2.95±0.45% of mRNA degradation (ns) (Figure 2b) due to the higher RNA fraction of the peak of non-degraded mRNA in the Moderna (147.26±17.80FU) than in the Pfizer-BioNTech (10.92±1.00FU) samples.

Discussion

A continued moderate movement at room temperature during 180 min of the reconstituted Pfizer-BioNTech and Moderna vaccines does not impair the mRNA's quality since this quality was similar without movement and did not differ from the original quality of the fresh samples. As expected, the mRNA in these reconstituted vaccines may be degraded under conditions reported to impair mRNA integrity [3], although the degradation after this massive shaking was only moderate, mainly with the Moderna vaccine. Therefore, exposure of the Pfizer-BioNTech and Moderna COVID-19 reconstituted vaccines to continuous movement mimicking ground transportation during 180 min at room temperature does not impair mRNA quality. Such a period of 180 min could guarantee ground transportation from 180 to 300 km in Europe and North America's road conditions. This distance would ensure the distribution of the vaccine after reconstitution to the institutions that do not have the appropriate facilities to do it. The permanence of the mRNA's integrity under these experimental conditions suggests

that the Pfizer-BioNTech and Moderna reconstituted vaccines and subsequently fractionated in syringes in pharmacy departments could be more easily distributed than initially expected.

Some limitations must be addressed. The total number of Moderna and Pfizer-BioNTech samples available was scarce since few samples were returned to the pharmacy department due to potential loss of microbiological traceability. This scarcity leads to a discrepant number of samples for the various arms to prioritize the groups with a lower range of changes induced by the independent variable (fresh, 180 min steady and swing) to optimize the detection of any possible modification due to this variable. Furthermore, no information is currently available about the possible direct consequences of RNA stability variation on alteration of vaccine efficacy. However, the prevention in the degradation of the active compound of the medication, the mRNA in these vaccines, represents a mandatory requirement.

In conclusion, the stability of Pfizer-BioNTech and Moderna reconstituted vaccines after continuous movement at room temperature may improve the efficiency in the administration of the vaccines, which may lead to shorter and more homogeneous vaccination in cities and rural areas. The possibility of preserving the mRNA after medium-distance ground transportation of reconstituted and fractionated syringes of both vaccines may optimize the distribution.

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Authors' contributions

All the authors contributed to the acquisition, analysis, and interpretation of data and to the critical revision of the manuscript. RM and SG are responsible of conceptualizing the research idea and design, making the final interpretation of the statistical analyses and writing the first draft and revision of the manuscript. EMG is responsible for the selection, realization and interpretation of statistical tests/analyses, and participated in writing the manuscript. OF participated in conceptualizing the research idea, making the primary interpretation of the statistical analyses and in writing the manuscript.

Transparency declaration

RM received in past 36 months grants or contracts from: Aelis, Angelini, Boehringer Ingelheim, Camurus, Esteve, GlaxoSmithKline, Grünenthal, Lundbeck, Pharmaleads, Sanofi, Spherium, Uriach.

SG received in past 36 months consulting fees from Pfizer and has been Advisor of Spanish Medicine Agency.

Funding

#ICREA Acadèmia2015 to R.M.

Acknowledgements

We are grateful to the Genomics Core Facility of University Pompeu Fabra for assistance with the mRNA integrity analysis. We thank R. Martín and J. Jancyte for their technical support. Figures with drawings are created with BioRender.com.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1. Analysis of mRNA integrity of reconstituted Pfizer-BioNTech COVID 19 vaccines. a) Measurement of the region of potentially degraded mRNA in fluorescence units (FU) of the samples immediately analyzed (fresh), or after 180 min without movement (180 min steady), continuous swing shaker movement (180 min swing), or Vortex shaking (180 min Vortex). Individual values of FU with the mean $\pm$ SEM are represented; one-way ANOVA, $F(3,38)=13.39$, $p<0.001$, Newman-Keus (*N-K*) post hoc test ***P < 0.001 Vortex vs the remaining groups (n=8-12). b) Percentage over the region's total area of potentially degraded mRNA of the samples immediately analyzed, or after 180 min without movement, continuous swing shaker movement, or Vortex shaking. Individual values of FU percentage with the mean $\pm$ SEM are represented; one-way ANOVA, $F(3,38)=5.772$, $p<0.01$, *N-K* post hoc test **P < 0.01 Vortex vs the remaining groups (n=8-12). c) Representative electropherograms expressed in FU of RNA integrity for different mRNA samples detailing the regions that indicate potentially degraded and intact mRNA peaks in the samples immediately analyzed, or after 180 min without movement, continuous swing shaker movement, or Vortex shaking.

Figure 2. Analysis of mRNA integrity of reconstituted Moderna COVID 19 vaccines. a) Measurement of the region of potentially degraded mRNA in fluorescence units (FU)

of the samples immediately analyzed (fresh), or after 180 min without movement (180 min steady), continuous swing shaker movement (180 min swing), or Vortex shaking (180 min Vortex). Individual values of FU with the mean ± SEM are represented; one-way ANOVA, $F(3,66)=14.77$, $p<0.001$, Newman-Keus (*N-K*) post hoc test ***P < 0.001 Vortex vs the remaining groups (n=14-23). b) Percentage over the region's total area of potentially degraded mRNA of the samples immediately analyzed, or after 180 min without movement, continuous swing shaker movement, or Vortex shaking. Individual FU percentage values with the mean ± SEM are represented; one-way ANOVA, $F(3,66)=1.745$, n.s. (n=14-23). c) Representative electropherograms expressed in FU of RNA integrity for different mRNA samples detailing the regions that indicate potentially degraded and intact mRNA peaks in the samples immediately analyzed, or after 180 min without movement, continuous swing shaker movement, or Vortex shaking.

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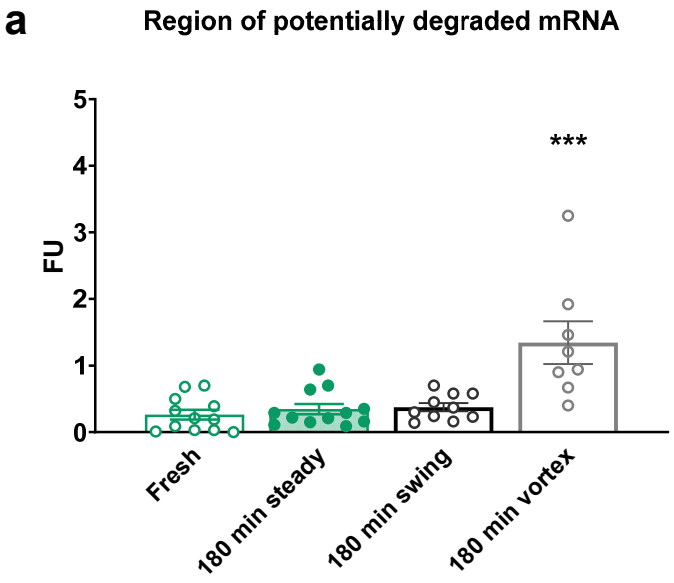

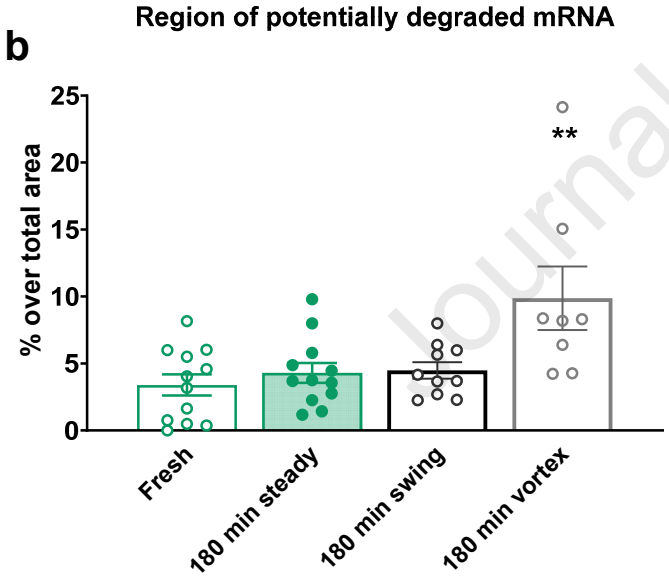

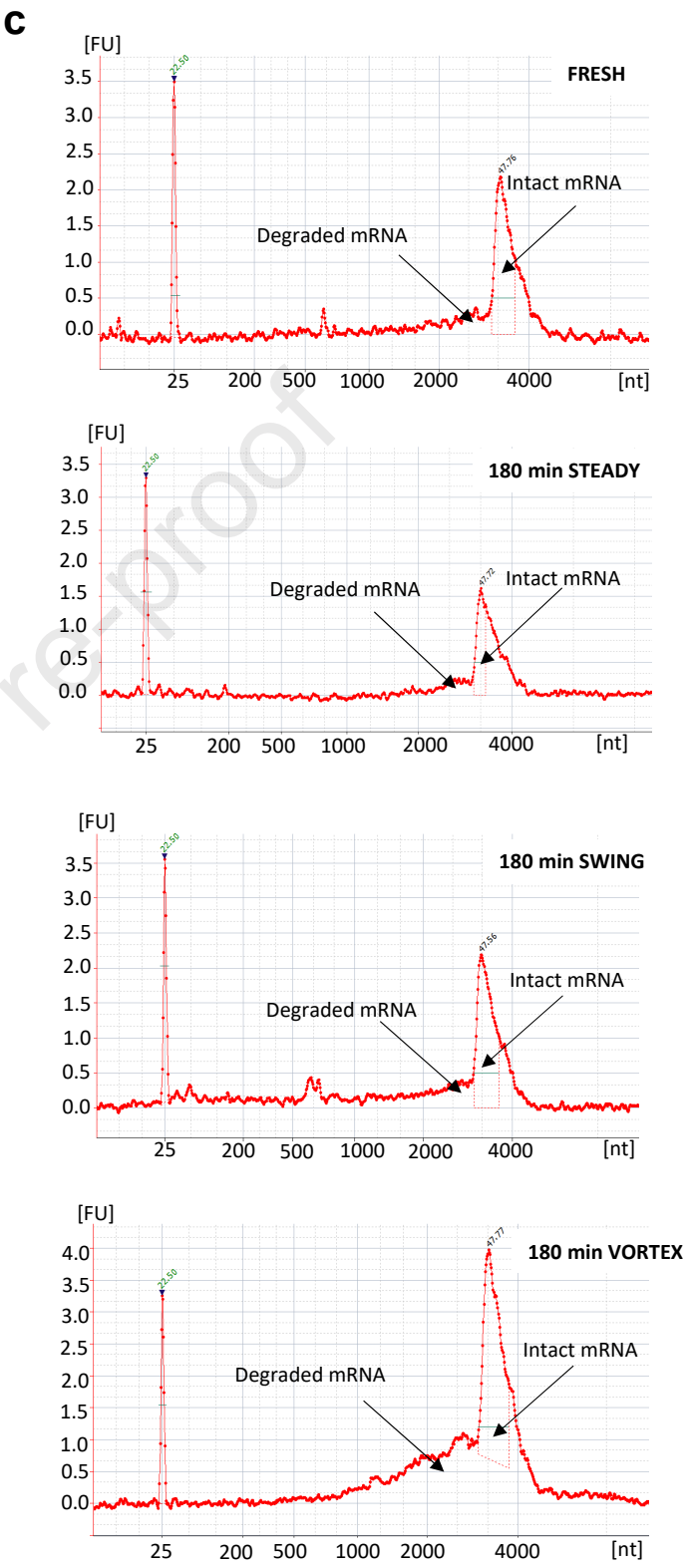

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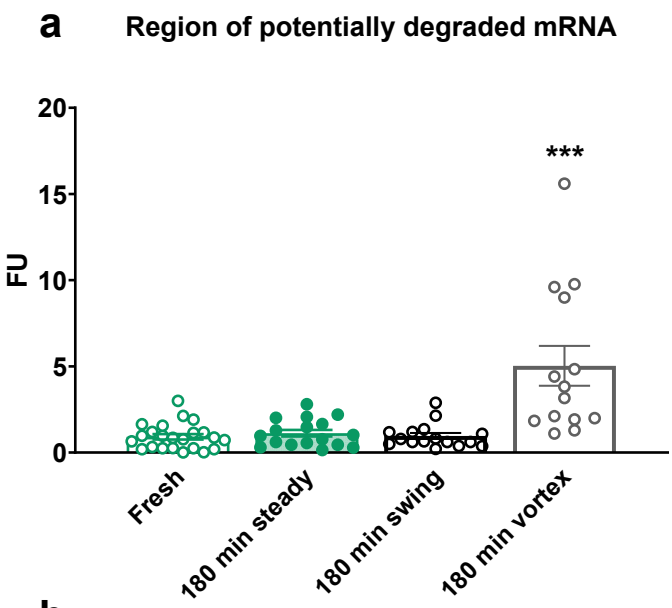

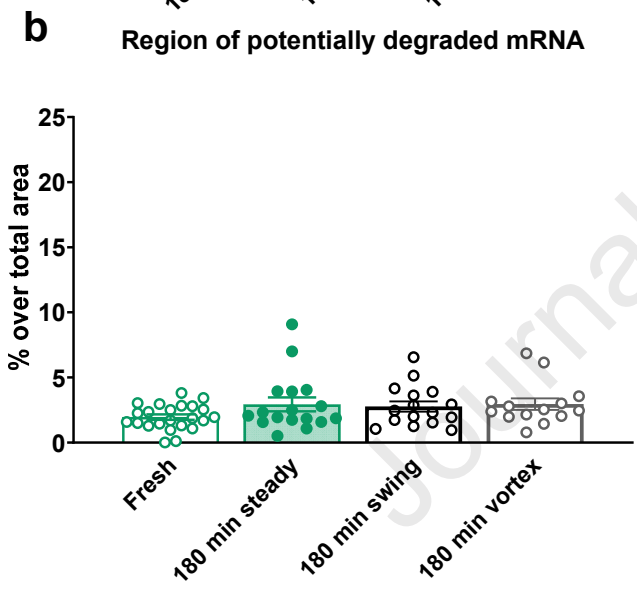

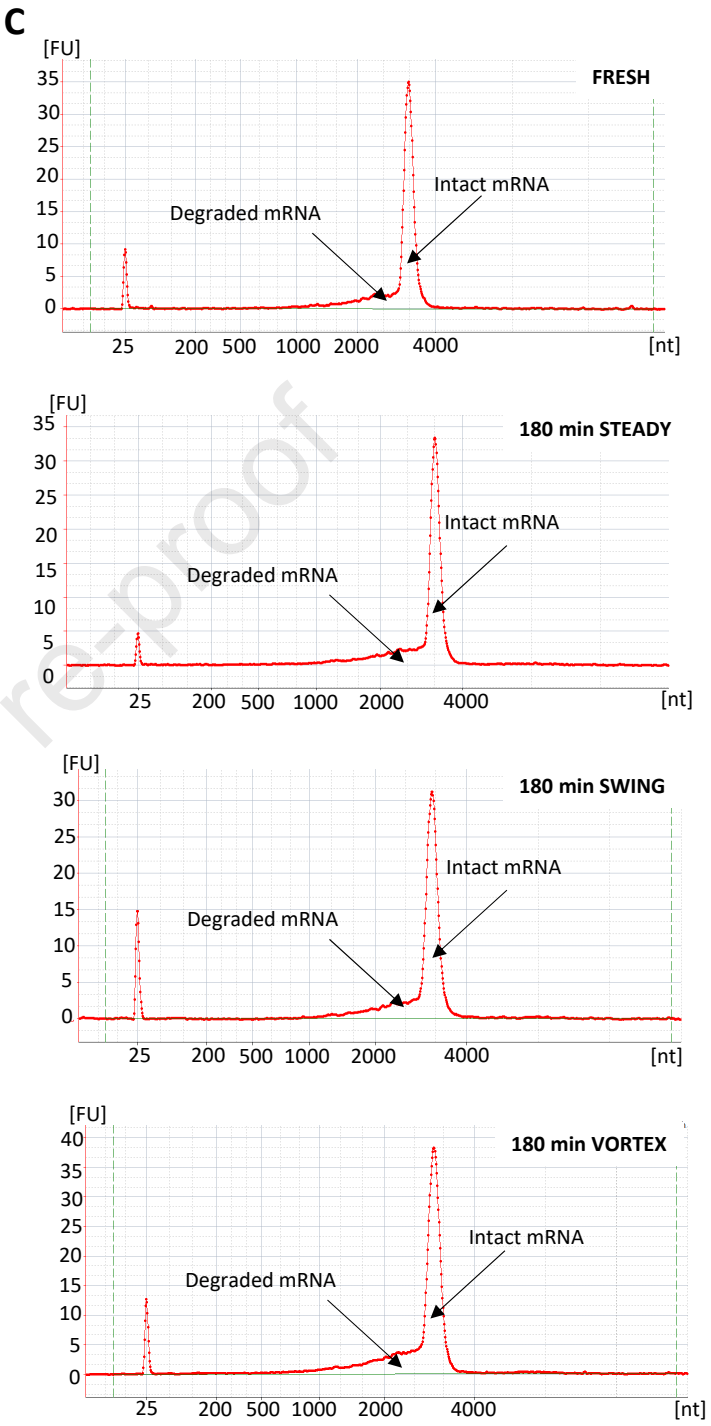

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