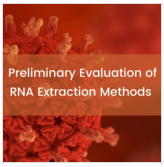

Jun 04, 2020

Preliminary Evaluation of RNA Extraction Methods

In 1 collection

Aniela Wozniak[1], Catalina Ibarra-Henriquez[2], Valentina Sebastian[3], Grace Armijo[2], Liliana Lamig[2], Carolina Miranda[3], Marcela Lagos[1], Sandra Solari[1], Ana María Guzmán[1], Teresa Quiroga[1], Susan Hitschfeld[2], Eleodoro Riveras[2], Marcela Ferres[1], Rodrigo A. Gutiérrez[2], Patricia García[1], Ariel Cerda[2]

[1]Departamento de Laboratorios Clínicos. Escuela de Medicina. Facultad de Medicina. Pontificia Universidad Católica de chile;
[2]FONDAP Center for Genome Regulation. Millennium Institute for Integrative Biology (iBio), Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, 8331150, Chile;
[3]Laboratorio de Microbiología. Servicio de laboratorios Clínicos. Red de Salud UC-CHRISTUS

1 *Works for me* This protocol is published without a DOI.

Coronavirus Method Development Community | Reclone.org (The Reagent Collaboration Network)

Catalina Ibarra-Henriquez
Pontificia Universidad Catolica de Chile

ABSTRACT

The technique RT-qPCR for viral RNA detection is the current worldwide strategy used for early detection of the novel coronavirus SARS-CoV-2. RNA extraction is a key pre-analytical step in RT-qPCR, often achieved using commercial kits. However, the magnitude of the COVID-19 pandemic is causing disruptions to the global supply chains used by many diagnostic laboratories to procure the commercial kits required for RNA extraction. Shortage in these essential reagents is even more acute in developing countries with no means to produce kits locally. We sought to find an alternative procedure to replace commercial kits using common reagents found in molecular biology laboratories. Here we report a method for RNA extraction that takes about 40 min to complete ten samples, and is not more laborious than current commercial RNA extraction kits. We demonstrate that this method can be used to process nasopharyngeal swab samples and yields RT-qPCR results comparable to those obtained with commercial kits. Most importantly, this procedure can be easily implemented in any molecular diagnostic laboratory. Frequent testing is crucial for individual patient management as well as for public health decision making in this pandemic. Implementation of this method could maintain crucial testing going despite commercial kit shortages.

EXTERNAL LINK

https://doi.org/10.1101/2020.05.07.083048

PROTOCOL CITATION

Aniela Wozniak, Catalina Ibarra-Henriquez, Valentina Sebastian, Grace Armijo, Liliana Lamig, Carolina Miranda, Marcela Lagos, Sandra Solari, Ana María Guzmán, Teresa Quiroga, Susan Hitschfeld, Eleodoro Riveras, Marcela Ferres, Rodrigo A. Gutiérrez, Patricia García, Ariel Cerda 2020. Preliminary Evaluation of RNA Extraction Methods. **protocols.io**
https://protocols.io/view/preliminary-evaluation-of-rna-extraction-methods-bghmjt46

EXTERNAL LINK

https://doi.org/10.1101/2020.05.07.083048

COLLECTIONS

A Simple RNA Preparation Method for SARS-CoV-2 detection by RT-qPCR

Copy of A Simple RNA Preparation Method for SARS-CoV-2 detection by RT-qPCR

KEYWORDS

Coronavirus, SARS-CoV-2, RNA extraction

and source are credited

CREATED

May 17, 2020

LAST MODIFIED

Jun 04, 2020

OWNERSHIP HISTORY

May 17, 2020	Liz Brydon	Protocols.io
Jun 03, 2020	Catalina Ibarra-Henriquez	Pontificia Universidad Catolica de Chile

PROTOCOL INTEGER ID

37133

PARENT PROTOCOLS

Part of collection
A Simple RNA Preparation Method for SARS-CoV-2 detection by RT-qPCR
Copy of A Simple RNA Preparation Method for SARS-CoV-2 detection by RT-qPCR

MATERIALS

NAME	CATALOG #	VENDOR
SDS	SB0485.SIZE.100g	Bio Basic Inc.
TRIZOL reagent	15596-026	Invitrogen - Thermo Fisher
100% EtOH	DSP-CA-151	Gold Shield Chemicals
Isopropanol	A464-4	Fisher Scientific
BSA	#A8806	Sigma Aldrich
EDTA	16 004Y	Fisher Scientific
Chloroform	372978	Sigma Aldrich
Nuclease-free Water (1.75 ml/tube)	AM9914G	Thermo Fisher
Sodium citrate dihydrate	W302600	Sigma Aldrich
Citric Acid Anhydrous	PHR1071	Sigma Aldrich
Sodium chloride (anhydrous free-flowing Redi-Dri ≥99%)	793566	Sigma Aldrich

MATERIALS TEXT

Biological samples
Obtain saliva samples from two asymptomatic volunteers.

RNA extraction methods evaluated
(1) TRIzol
TRIzol
chloroform
isopropanol
70% ethanol
RNAse-free water

(2) BSA-based method
1 mg/mL BSA solution

(3) Acid pH-based method
Lysis Buffer
20 g/L sodium dodecyl sulfate (SDS)
20 g/L sodium citrate dihydrate
25.36 g/L anhydrous citric acid
10 mM EDTA
Precipitation Buffer

5 g/L sodium citrate dihydrate
6.4 g/L anhydrous citric acid
234 g/L anhydrous NaCl
isopropanol
70% ethanol (cold)
Nuclease-free water (pre-warmed at 70°C)

(4) High temperature-based method
BSA (20 mg/mL)

(5) Direct use of the samples

Machines required
Step-One thermal cycler (Applied Biosystems)

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards. Obtain all necessary approvals from relevant Ethics Committees.

Biological Samples

1 Obtain **two saliva** samples from each asymptomatic volunteer and perform at least three independent RNA extractions from each sample, obtaining a minimum of six RNA preparations to test each experimental procedure.

Two types of biological samples were used:
1. For preliminary evaluation of the RNA extraction methods, use saliva samples obtained from two asymptomatic volunteers.
2. For validation of the RNA extraction method selected, use nasopharyngeal swabs in Universal Transport Medium (UTM). (This protocol can be found here: Validation of Selected RNA Extraction Method)

Saliva is routinely collected for the initial assessment of viral infection.

RNA Extraction

2 Centrifuge saliva samples before taking an aliquot of supernatant for processing using the acid pH-based method.

Under acidic pH, RNA can be separated from DNA and other molecules due to the differential polarity given by its hydroxyl groups which maintains it in solution.

Based on the methods described in the following protocols:

Heath, E. Low pH RNA isolation reagents, method, and kit. USA patent (1999).

Sambrook, J. & Russell, D. Molecular cloning: a laboratory manual. 3rd edn, (Cold Spring Harbor Laboratory, 2001).

Chomczynski, P. & Sacchi, N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nature protocols 1, 581-585, doi:10.1038/nprot.2006.83 (2006).

3 Add **300 µl Lysis Buffer, pH 5** to **200 µl uncentrifuged sample** and mix by pipetting 3 times.

Lysis Buffer contains:
20 g/L sodium dodecyl sulfate (SDS)
20 g/L sodium citrate dihydrate
25.36 g/L anhydrous citric acid
10 mM EDTA

4 Add **150 µl Precipitation Buffer** and mix by inversion 10 times.

Precipitation Buffer contains:
5 g/L sodium citrate dihydrate
6.4 g/L anhydrous citric acid
234 g/L anhydrous NaCl

5 Incubate samples **On ice** for **00:05:00** .

6 Centrifuge samples at **15000 x g, Room temperature 00:03:00** .

7 Transfer **600 µl supernatant** to a clean tube containing **600 µl isopropanol** and incubate for **00:10:00** at **Room temperature** .

8 Centrifuge samples at **15000 x g, Room temperature 00:05:00** .

9 Discard the supernatant.

10 Wash the pellet with **300 µl cold 70% ethanol** .

11 Centrifuge at **15000 x g, Room temperature 00:03:00** .

12 Discard the supernatant and invert tubes in paper towel.

13 Dry the pellet by leaving tubes open for **00:10:00** .

14 Resuspend pellet in **50 µl nuclease-free water (pre-warmed at 70°C)** .

RT-qPCR Analysis

15 Use RT-qPCR against the human RNAseP gene with primers and a Taqman probe as previously described.

Waggoner, J. J. et al. (2013). Development of an internally controlled real-time reverse transcriptase PCR assay for pan-dengue virus detection and comparison of four molecular dengue virus detection assays.. Journal of clinical microbiology.
http://doi:10.1128/jcm.00548-13

15.1 Add **2 µl RNase-Free water** .

15.2 Add **10 µl 2X TaqMan Fast Universal PCR Master Mix** .

15.3 Add **1 µl EACH RNase P primer** .

15.4 Add **1 µl TaqMan RNase P probe** .

15.5 Add **5 µl RNA from saliva sample** .

16 In a final reaction volume of 20 µl, perform RT-qPCR in a Step-One thermal cycler (Applied Biosystems).