Microwaves: a Novel Approach to SARS-CoV-2 Genome Extraction

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Abstract

On this study an innovative approach to the heat extraction has been tested: the use of microwaves, which can decrease dramatically the time needed to do the genome extraction. The method can obtain the virus with enough quality to assure the identification by q(RT)-PCR and minimize procedures and contaminations.

Main Text

With the global SARS-CoV2 pandemic, the need of quick and efficient techniques to process samples has become an aim for all the clinical laboratories around the world. Several methods like heat extraction can be a clear alternative to improve times of procedure at the time of doing the genome extraction (1–4).

On this study an innovative approach to the heat extraction has been tested: the use of microwaves, which can decrease dramatically the time needed to do the genome extraction. The method can obtain the virus with enough quality to assure the identification by q(RT)-PCR and minimize procedures and contaminations.

To check this hypothesis, a set of 43 nasopharyngeal samples were extracted by five different methods: two of them using chaotropic reagents, and three based on heat extraction. The two methods that use reagents were automatic MagNa Pure 96 (Roche, Ginebra) taken as reference, and the “Bikop” method developed on our laboratory previously (3). Both procedures were carried with 200µL of each sample.

About the three methods based on heat extraction, one consistent just in a heat application at 98°C for 10 minutes as the normal method. Second method was based on the addition of proteinase K (pK) to favour denaturalization before heat application; for this method, the heat application had two steps: first at 56°C during 5 minutes followed by other at 98°C for 10 minutes (5–7). The last one method of the heat extraction was the use of microwaves. This method was performed on a microwave oven Schneider SMW205 (Schneider Electric, Llanera, Spain), during 1 minute at 800W and 2450MHz of microwave frequency. For all these protocols, the heat application was followed by a cooling step on ice for 5 minutes. A volume of 100µL of each sample were used.

All extracted samples were tested with a multiple qRT-PCR directed to two regions of the SARS-CoV 2 genome (Orf1ab and N gene). Briefly, 5µl of sample, previously extracted by any of the tested methods, were added to 10µl of TaqMan Fast 1-Step Master Mix (Life technologies, Carlsbad, CA) supplemented with a mixture of primers (Thermo Fisher Scientific, Waltham, MA) and taqman MGB probes (Applied Biosystems, Foster City, CA) which were the same conditions as used in reference 3. *Amplification and subsequent analysis were carried out using the Applied Biosystems 7500 Real-time PCR System (Applied Biosystems). The cycling protocol was as follows: (50°C, 20 min; 95°C, 5 min; 45 cycles of 95°C, 10 sec; 55°C, 15 sec and 60°C, 30 sec).*

The cycle threshold for each sample on each procedure and the statistical results, like sensitivity, of the total and broken down into less than and greater or equal to Ct 30 are shown in tables 1 and 2.

These data show that all the protocols tested are over 95% of sensitivity, except for the microwaves method, that almost get 90%. Despite this exception, differences are not significant.

Viral load, reflected as Ct, has influence on these results: when a threshold was made in Ct, all procedures analysed were sensitive enough for samples which were amplified under 30 cycles, with results over 95% for every method. Over 30 Ct, or even more, over 32 Ct, a decrease in sensitivity happens, especially on microwaves method. As amplification of these samples can be confuse according to their capacity to infect, results over this Ct should be taken in care in any case. On the other hand, it is noted that manual methods have higher variance than automatized reference methods, as ranges of each sample are higher than in MP96 method. Special care must be taken when manual methods are performed.

In conclusion, the results confirm that heat extraction can be implemented in a clinical laboratory as a fast method to get samples ready for the q(RT)-PCR and offer a sooner detection. The use of microwaves method can be the fastest way of extraction when the number of samples exceed the capacity of the laboratory to process them in time, specially when high incidence is noted, in order to prevent the elongation of the contagious chain.

Declarations

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Declaration of Interest Statement

Authors declare no potential conflicts of interest.

Ethical Committee

This study was approved by Comité de Ética de la Investigación del Principado de Asturias with code CEImPA 2021.188

References

1. Merindol N, Pépin G, Marchand C, et al. SARS-CoV-2 detection by direct rRT-PCR without RNA extraction. J Clin Virol. 2020;128:104423. doi:10.1016/j.jcv.2020.104423
2. Mancini F, Barbanti F, Scaturro M, et al. Laboratory management for SARS-CoV-2 detection: a user-friendly combination of the heat treatment approach and rt-Real-time PCR testing. Emerg Microbes Infect. 2020;9(1):1393-1396. doi:10.1080/22221751.2020.1775500

3. Martín G, Rojo-Alba S, Castelló-Abietar C, et al. Comparison of in-house SARS-CoV-2 genome extraction procedures. A need for COVID-19 pandemic. Research Square preprint 2021 Mars 15. doi:10.21203/rs.3.rs-214704/v1
4. Smyrlaki, I., Ekman, M., Lentini, A. *et al.* Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. Nat Commun 11, 4812 (2020). doi:10.1038/s41467-020-18611-5
5. García F, Melón S, Navarro D, et al. Organización del diagnóstico de SARS-CoV-2 y estrategias de optimización. SEIMC protocol 14/10/2020
<https://seimc.org/contenidos/documentoscientificos/recomendaciones/seimc-rc-2020-COVID19-OrganizacionDiagnostico.pdf>
6. Mallmann L, Schallenberger K, Demolliner M, et al. Pre-treatment of the clinical sample with Proteinase K allows detection of SARSCoV-2 in the absence of RNA extraction. bioRxiv preprint 2020 May 9. doi: 10.1101/2020.05.07.083139
7. Vogels CBF, Brackney DE, Wang J, et al. SalivaDirect: Simple and sensitive molecular diagnostic test for SARS-CoV-2 surveillance. medRxiv preprint. 2020 Aug 4. doi:10.1101/2020.08.03.20167791

Tables

Table 1
Sensitivity, mean, range, IC95% and p-value for the different methods tested

	Extraction with reagents		Heat Extraction		
	MP96	Bikop	Normal	With pK	Microwaves
Total					
Positives	43 (100%)	41 (95,34%)	41 (95,34%)	41 (95,34%)	38 (88,37%)
$\bar{x} \pm \sigma$	29,07 $\pm$ 4,44	27,29 $\pm$ 4,36	26,39 $\pm$ 4,57	25,58 $\pm$ 4,02	27,31 $\pm$ 5,02
Range	(21–34)	(15–37)	(17–36)	(18–35)	(17–37)
IC95%	(27,86 – 30,28)	(25,99 – 28,59)	(25,03–27,75)	(24,38 – 26,78)	(25,81 – 28,81)
p value	-	0,20	0,21	0,21	0,04
Ct < 30					
Positives	21 (100%)	20 (95,24%)	21 (100%)	21 (100%)	20 (95,24%)
$\bar{x} \pm \sigma$	25,57 $\pm$ 2,68	25,15 $\pm$ 4,30	24,14 $\pm$ 4,22	24,85 $\pm$ 4,14	26,3 $\pm$ 5,39
Range	(21–29)	(15–32)	(17–31)	(18–30)	(17–35)
IC95%	(24,43 – 26,71)	(23,31 – 26,99)	(22,33 – 25,95)	(23,08–26,62)	(23,99 – 28,61)
p value	-	0,23	0,45	0,49	0,45
Ct $\geq$ 30					
Positives	22 (100%)	21 (95,45%)	20 (90,91%)	20 (90,91%)	18 (81,82%)
$\bar{x} \pm \sigma$	32,4 $\pm$ 1,37	29,33 $\pm$ 3,40	28,75 $\pm$ 3,70	26,35 $\pm$ 3,86	28,44 $\pm$ 4,45
Range	(30–34)	(23–37)	(22–36)	(21–35)	(20–37)
IC95%	(31,83 – 32,97)	(27,91 – 30,75)	(27,20–30,30)	(24,74 – 27,96)	(26,58 – 30,30)
p value	-	0,19	0,09	0,10	0,02

Table 2
Cycle threshold for each sample by genome extraction procedure

Sample	Extraction with reagents		Heat Extraction		
	MP96	Bikop	Normal	With pK	Microwaves
1°	21	27	21	22	19
2°	21	20	17	18	17
3°	23	15	18	21	21
4°	23	21	24	30	29
5°	23	28	26	30	26
6°	23	19	18	18	18
7°	24	25	23	27	32
8°	24	27	30	30	27
9°	25	25	23	27	31
10°	25	27	23	29	29
11°	25	29	30	24	24
12°	26	25	23	29	29
13°	27	25	21	21	35
14°	27	30	28	26	32
15°	28	31	29	24	0
16°	28	25	29	23	34
17°	28	22	21	21	22
18°	29	22	21	21	24
19°	29	0	25	30	26
20°	29	28	26	22	21
21°	29	32	31	29	30
22°	30	26	28	21	24
23°	30	23	23	22	26
24°	30	28	26	24	25
25°	31	28	0	29	31

	Extraction with reagents		Heat Extraction		
26°	31	32	29	27	26
27°	31	31	32	35	30
28°	32	27	31	27	35
29°	32	29	31	32	37
30°	32	26	22	23	25
31°	33	31	0	28	0
32°	33	32	25	22	20
33°	33	0	35	28	27
34°	33	27	26	23	31
35°	33	25	30	22	28
36°	33	30	29	0	28
37°	33	27	26	32	26
38°	33	31	32	26	26
39°	34	29	36	0	0
40°	34	31	28	25	31
41°	34	37	28	26	36
42°	34	36	32	30	0
43°	34	30	26	25	0