RESEARCH ARTICLE

Guthrie Cards: Exploring Their Use for Nasopharyngeal Sample Storage

Gabriel Martín*[,1,2], Ana Isabel Fidalgo[2], Roberto Prada[2], Reyes Carvajal[2], Virginia Linares-Fernández[2], Carmen González-Nieto[2], María José Ferrero[2], Rainer Campo-Ramos[1,2], Isabel Cos tales[1,2]

[1]Grupo de Microbiología Traslacional, Instituto de Investigación Sanitaria del Principado de Asturias (Oviedo, Asturias; Spain)
[2]Servicio de Microbiología, Hospital Universitario Central de Asturias (Oviedo, Asturias; Spain)

*Corresponding author: gabrielmartinrguez1994@gmail.com

ABSTRACT
With SARS-CoV-2 pandemic, a great amount of samples have been received on the different laboratories for diagnosis, sometimes in amounts difficult to handle in time, with the associated stability and quality risks.
So this study analyses the possibility to use Guthrie cards of cellulose for the storage of nasopharyngeal samples from patients with a respiratory virus infection.
With this aim, a total of 70 samples obtained on the classic UTM tube for sample collection from different patients were replicated on Guthrie cards; then, genome extraction and amplification for different viruses was performed and compare to stablish detection when samples are recovered from the cards.
Results show detection percentages of 66% for SARS-CoV-2, 75% for Picornavirus, 100% for Respiratory Syncytial Virus and 50% for Adenovirus after one week of storage.

OPEN ACCESS

Published: August 31, 2023

Citation: Martín G, Fidalgo AI, et al., 2023. Guthrie Cards: Exploring Their Use for Nasopharyngeal Sample Storage, Medical Research Archives, [online] 11(8). https://doi.org/10.18103/mra.v11i8.4111

DOI
https://doi.org/10.18103/mra.v11i8.4111

ISSN: 2375-1924

Introduction

Since the spread of SARS-CoV-2 on 2019 to the end of the Public Health Emergency declaration realised by the WHO on May 2023[1-2], the number of confirmed cases reported reach 95,139.14 million around the world[3]. This, obviously, has meant a huge number of samples received by the different hospitals.

The classical way to collect these samples is the use of swabs in universal transport medium (UTM) tubes, with no difference in the accuracy of viral detection between nasopharyngeal and oropharyngeal samples[4].

But the storage of this kind of samples can be a problem during emergency situations like during pandemic, when the great amount of them can saturate the diagnostic capacity of the laboratory, delaying the analysis of the samples, something that can affect the viral load on them by multiple factors[5].

So, the enlargement of storage and sample collection methods could be an interesting way to relieve pressure on points-of-care and other sample collection places.

Based on an old method for sample collection, Guthrie cards, developed for their use to phenylketonuria detection by Guthrie test[6], but also used for diagnosis of a small number of virus circulating in the blood[7-10], this study explores their use for nasopharyngeal sample storage.

Material and Methods

- ***Samples:*** a set of 70 nasopharyngeal samples were collected in the Hospital Universitario Central de Asturias, on UTM collection tubes. Of the volume collected (1.5mL), one part (200µL) was extracted by the routine method on the lab, the MagNa Pure 96 system (Roche; Ginebra) and other part (100µL) was added to a clean Guthrie card and was left till total dry.

- ***Guthrie cars storage:*** after their total dry, this kind of samples were stored for 1 week at room temperature in a drower type card holder, using silica gel to avoid humidity.

- ***Guthrie card samples preparation:*** a circle of 1cm diameter of each dry Guthrie cards (ARCHIMEDlife; Vienna, Austria) sample was cut and incubated on 200µL of Minimal Essential Medium (MEM) (Dominique Dutscher, Brumath, France) with 50µL of proteinase K (pK) for 1 hour at 56°C, to extract the viral particles from the cellulose matrix of the filter. After incubation, the total volume was extracted by the "Bikop" method, developed by our team, and exposed on previous articles[11,12].

- ***Genome detection:*** all extracted samples from UTM tubes and Guthrie cards, were tested with a RT-qPCR, directed against different respiratory virus genomes, using 5µl of the sample, previously extracted by any of the tested methods. This volume was added to 6µl of TaqMan Fast 1-Step Master Mix (Life technologies, Carlsbad, CA) with 4µl of the adequate mixture of primers (Thermo Fisher Scientific, Walthman, MA) and taqman MGB probes (Applied Biosystems, Foster City, CA) (Table 1).

  Amplification and subsequent analysis were carried out using the Applied Biosystems 7500 Real-time PCR System (Applied Biosystems). The cycling protocol was, in all cases, 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).

Results

On the different samples analysed a variety of respiratory virus was searched, being detected the followed: SARS-CoV-2, Picornavirus, Respiratory Syncytial Virus (RSV) and Adenovirus (ADV). Detection results for all these viruses, including mean, range and IC 95% are shown on Table 2, exposed by cycle threshold and viral copies (log)/mL. Same data but for the SARS-CoV-2 positive samples are shown on Table 3.

On the Guthrie cards, the following samples for the 4 different viruses detected were: 33 (66%) for SARS-CoV-2, 6 (75%) for Picornavirus, 4 (100%) for RSV and 1 (50%) for ADV.

When SARS-CoV-2 samples were analysed closely, the following samples were detected by cycle threshold ranges: 12 (60%) for Ct under 23, 12 (66.67%) for Ct between 23 and 29 and 9 (75%) for cycles over 30.

On the other hand, when they were analysed by viral load, expressed as viral copies (log)/mL, the following samples were detected: 23 (62.16%) for viral loads over 5 log, 5 (71.43%) for viral loads between 4 anf 4.99 log, and 5 (83.33%) for viral loads under 4 log.

Discussion

Even when it is the classical and most recommended method for sample collection, specially when viral detection is involved, Universal Trasport Medium (UTM) tubes have a short expiration date after their use for sample collection.

As Hosokawa-Muto et al (2014) have reported, different factors, like time, temperature and bacteria presence can affect the viral load collected, always decreasing it, being able to make it even undetectable[5]. Something than, as has been

said before, can be a significant problem during special situations when work piles up.

So, to propose an alternative for the storage this study analysed the use of Guthrie cards.

In general, after results obtained, we can affirm that a proper genome extraction of different virus can be performed from Guthrie cards, as we had obtained an amplification on 68.75% of the positive samples tested. Specifically, sensitivity for the different virus detected was: 33 (66%) for SARS-CoV-2, 6 (75%) for Picornavirus, 4 (100%) for RSV and 1 (50%) for ADV.

The low number of samples of no SARS-CoV-2 virus detected represent a clear bias for the analysis, but the good results found by better analysing the SARS-CoV-2 results lead us to minimize it

Focusing on these, which are the longest cohort of the samples, obviously for the actual situation, we see that 2 of each 3 extracted samples were detected after 1 week on Guthrie cards, with sensitivities upper 60% when we look the ranges analysed.

Moreover, when we consider ranges where the number of viral copies is lower ($Ct \geq 30$ and $\log \leq 3{,}99$) we obtained good sensitivities (75% and 83.33% respectively). And, even when the number of positive samples for other virus is short, they show a similar tendency.

On the other hand, results for SARS-CoV-2 show higher Ct/lower viral loads for Guthrie card samples than UTM for the ranges corresponding with the lowest amount of virus expected. This may have been caused by the concentration involved in the processing for the extraction of the Guthrie card samples, but not affect the results.

It should be said that the proposal to use this support as a genome bank was already made in 1994 by McEwen and Reilly[14], in addition to considering the traditional use of this support for the detection of viruses present in the blood found in previous studies as: Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), ADV or Citomegalovirus (CMV)[7-10].

So, with these facts and the results obtained, especially considering the time between preparation of samples on Guthrie cards and their processing for extraction and genome detection, something that has been validated before, we propose their use as a practical way of storage big numbers of viral samples, always taking care of humidity and temperature control.

Of course, more studies should be done to find the limits of this method, but now, it presents two great advantages over UTM tubes: one, their small size, so they can be storage in larger amounts, and two, the greater biodegradability of cellulose compared to the plastic UTM tubes[15].

On the other hand, their use for self-collection is a way that can be explored, as for this assay we start from collected nasopharyngeal samples, but sample collection on Guthrie cards will avoid the need for specialized workers and the massifications of sick people at the points-of-care for sample collection, or it can even be a solution when UTM are out of stock, as it was at the beginning of this pandemic, as American Society of Microbiology predicted[16].

Conclusions

1. Guthrie cards are a viable method for nasopharyngeal samples storage.
2. Guthrie cards can storage different respiratory virus (SARS-CoV-2, Picornavirus, RSV and ADV) for at least a week.

Declarations

- ***Funding:*** Not applicable.
- ***Conflicts of interest / Competing interests:*** The authors declare no potential conflicts of interest or competing interests.
- ***Ethics approval:*** This study was approved by Comité de Ética de la Investigación del Principado de Asturias with code CEImPA 2021.188.
- ***Authors' contributions:*** All authors have contributed equally to this paper.
- ***Consent for publication:*** All authors have expressed their consent for the publication of this paper.

Bibliography

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W, China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Feb 20;382(8):727-733. doi: 10.1056/NEJMoa2001017.
2. Web page of the WHO (World Health Organisation) https://www.who.int/europe/emergencies/situations/covid-19 (consulted on 23/06/2023).
3. Ritchie H, Mathieu E, Rodés-Guirao L, Appel C, Giattino C, Ortiz-Ospina E, Hasell J, Macdonald B, Beltekian D, Roser M. Coronavirus Pandemic (COVID-19). OurWorldInData.org. [On-line]. Available at: https://ourworldindata.org/covid-cases. (consulted on 23/06/2023).
4. Manohar C, Sun J, Schlag P, et al. Agile design and development of a high throughput cobas SARS-CoV-2 RT-PCR diagnostic test. Heliyon. 2022;8(9):e10591. Published 2022 Sep 11. doi:10.1016/j.heliyon.2022.e10591
5. Hosokawa-Muto J, Fujinami Y, Mizuno N. Evaluation of the Universal Viral Transport system for long-term storage of virus specimens for microbial forensics. J Forensic Leg Med. 2015;34:29-33. doi:10.1016/j.jflm.2015.04.019
6. Guthrie R, Whitney S. Phenylketonuria; detection in the newborn infant as a routine hospital procedure, State University of New York, Buffalo, 1964 (Children's Bureau Publication No. 419, 1964)
7. Comeau AM, Hsu HW, Schwerzler M, et al. Identifying human immunodeficiency virus infection at birth: application of polymerase chain reaction to Guthrie cards. J Pediatr. 1993 Aug;123(2):252-8. doi: 10.1016/s0022-3476(05)81697-x.
8. Barbi M, Binda S, Primache V, et al. Use of Guthrie cards for the early diagnosis of neonatal herpes simplex virus disease. Pediatr Infect Dis J. 1998 Mar;17(3):251-2. doi: 10.1097/00006454-199803000-00017.
9. Honkaniemi E, Talekar G, Huang W, et al. Adenovirus DNA in Guthrie cards from children who develop acute lymphoblastic leukaemia (ALL). Br J Cancer. 2010 Mar 2;102(5):796-8. doi: 10.1038/sj.bjc.6605581.
10. Pellegrinelli L, Alberti L, Pariani E, Barbi M, Binda S. Diagnosing congenital Cytomegalovirus infection: don't get rid of dried blood spots. BMC Infect Dis. 2020 Mar 12;20(1):217. doi: 10.1186/s12879-020-4941-z.
11. 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, Journal of Virological Methods (2021), doi:10.1016/j.jviromet.2021.114415
12. Álvarez-Argüelles, M.E., Rojo-Alba, S.; Martín, G. et al. Utilization of Microwaves: A Novel Approach to SARS-CoV-2 Genome Extraction. Appl. Microbiol. 2022, 2, 800–804. doi:10.3390/applmicrobiol2040060
13. Web page of the CDC (Centre for Diseases Prevention and Control) https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html (consulted on 23/06/2023).
14. McEwen JE, Reilly PR. Stored Guthrie cards as DNA "banks". Am J Hum Genet. 1994;55(1):196-200.
15. Erdal NB, Hakkarainen M. Degradation of Cellulose Derivatives in Laboratory, Man-Made, and Natural Environments. Biomacromolecules. 2022;23(7):2713-2729. doi:10.1021/acs.biomac.2c00336
16. American Society for Microbiology. Statemente. ASM Expresses Concern about Test Reagent Shortages. [On-line]. Available at: https://asm.org/Articles/Policy/2020/March/ASM-Expresses-Concern-about-Test-Reagent-Shortages. (Consulted on 23/06/2023).

Table 1: Primers and probes used for the different RT-qPCR

Design	Target	Gen	Function	Name	Sequence (5´-3´)
In-house	SARS-CoV-2	ORF1ab	Forward primer	CoV-2-OVI-S	ATCAAGTTAATGGTTACCCTAACATGT
			Reverse primer	CoV-2-OVI-A	AACCTAGCTGTAAAGGTAAATTGGTACC
			MGB probe FAM	CoV-2-OVI-FAM	CCGCGAAGAAGCTA
CDC[1]	SARS-CoV-2	N	Forward primer	2019-nCoV-N1-F	GACCCCAAAATCAGCGAAAT
			Reverse primer	2019-nCoV-N1-R	TCTGGTTACTGCCAGTTGAATCTG
			Sonda MGB VIC	2019-nCoV-N1-P-VIC	CCGCATTACGTTTGGT[2]
In-house	Picornavirus	P2	Forward primer	Picor-Ext-1 mod	GCCCCTGAATGYGGCTAA
			Reverse primer	Picor-Int-4 mod	GAIACYTGWGCICCCAT
			MGB probe VIC	Picorna-VIC	ACTTTGGGTGTCCGTGTT
In-house	RSV	F	Forward primer	VSRA-TR-S	GCCAGTGGCATTGCTGTAT
			Reverse primer	VSRA-TR-A	CTGACTACGGCCTTGTTTGT
			Forward primer	VSRB-TR-S	GCAAGTGGTATAGCTGTAT
			Reverse primer	VSRB-TR-A	CTGACTACAGCTTTGTTTGT
			MGB probe VIC	VSR-VIC	GAAGTGAACAARATCAA
In-house	ADV	Hexon	Forward primer	ADV2-TR-S	CCAGGACGCCTCGGAGTA
			Reverse primer	ADV2-TR-A	AAACTTGTTATTCAGGCTGAAGTACGT
			Sonda MGB NED	ADV2-NED	AGTTTGCCCGCGCCACCG
			Forward primer	ADV4-TR-S	GGACAGGACGCTTCGGAGTA
			Reverse primer	ADV4-TR-A	CTTGTTCCCCAGACTGAAGTAGGT
			MGB probe NED	ADV4-NED	CAGTTCGCCCGYGCMACAG
In-house	Human genome	β-globina	Forward primer	Beta-TR-S	ACACAACTGTGTTCACTAGC
			Reverse primer	Beta-TR-A	CCAACTTCATCCACGTTCACC
			MGB probe Cy5	Beta-Cy5	TGCATCTGACTCCTGAGGA

[1] Sequences published by Center for Disease Control and Prevention (CDC)[13]

[2]Probe sequence has been shortened as it is a MGB probe

Table 2: Sensitivity, average, range, and IC 95% for all the samples, expressed by Ct (above) and normalized viral load (below).

		UTM samples			***Guthrie cards***				
	N	$\bar{X} \pm \sigma$	*Range*	*IC95%*	*Sensitivity*	$\bar{X} \pm \sigma$	*Range*	*IC95%*	*p-value (0.05)*
SARS-CoV-2	50	25.64 ± 5.17	(18 - 37)	(24.21 - 27.07)	33 (66.00%)	27.15 ± 4.75	(18 - 36)	(25.53 - 28.77)	0.0022
Picornavirus	8	23.25 ± 3.20	(17 - 26)	(21.04 - 25.46)	6 (75.00%)	26.00 ± 6.16	(20 - 36)	(21.07 - 30.93)	0.0208
RSV	4	30.50 ± 7.59	(21 - 38)	(22.61 - 37.49)	4 (100%)	27.00 ± 5.03	(20 - 32)	(22.07 - 31.93)	0.0494
Adenovirus	2	28	(22 - 34)	-	1 (50%)	25*	-	-	-
Total	64	25,72 ± 5.31	(17 - 37)	(24.42 - 27.02)	44 (68,75%)	26.93 ± 4.82	(18 - 36)	(25.51 - 28.35)	0.0051

		UTM samples			***Guthrie cards***				
	N	$\bar{X} \pm \sigma$	*Range*	*IC95%*	*Sensitivity*	$\bar{X} \pm \sigma$	*Range*	*IC95%*	*p-value (0.05)*
SARS-CoV-2	50	6.52 ± 1.86	(2.92 - 11.12)	(6.00 - 7.04)	33 (66.00%)	6.58 ± 1.41	(3.95 - 9.31)	(6.10 - 7.06)	0.0003
Picornavirus	8	7.41 ± 1.32	(5.07 - 9.52)	(6.50 - 8.32)	6 (75.00%)	6.93 ± 1.84	(3.95 - 8.72)	(5.46 - 8.40)	0.0025
RSV	4	4.95 ± 1.44	(3.26 - 6.74)	(3.54 - 6.36	4 (100%)	6.63 ± 1.50	(5.15 - 8.72)	(5.16 - 8.10)	0.0442
Adenovirus	2	5.61	(4.68 - 6.55)	-	1 (50%)	7.23*	-	-	-
Total	64	6.50 ± 1.82	(2.92 - 11.12)	(6.05 - 6.95)	44 (68,75%)	6.65 ± 1.44	(3.95 - 9.31)	(6.23 - 7.07)	0.5223

*This is the value for the one positive value

Table 3: Sensitivity, average, range, and IC 95% for the SARS-CoV-2 positive samples, expressed by Ct (above) and normalized viral load (below) ranges.

		UTM samples			***Guthrie cards***				
	N	$\bar{X} \pm \sigma$	*Range*	*IC95%*	*Sensitivity*	$\bar{X} \pm \sigma$	*Range*	*IC95%*	*p-value (0.05)*
Ct ≤ 23	20	20.60 ± 1.57	(18 - 23)	(19.91 - 21.29)	12 (60.00%)	26.91 ± 6.04	(18 - 36)	(24.26 - 29.56)	< 0.0001
24 ≤ Ct ≤ 29	18	26.33 ± 1.75	(24 - 29)	(25.52 - 27.14)	12 (66.67%)	26.25 ± 4.73	(19 - 36)	(24.06 - 28.44)	0.0002
Ct ≥ 30	12	33.00 ± 2.00	(30 - 37)	(31.87 - 34.13)	9 (75.00%)	28.67 ± 2.35	(25 - 32)	(27.34 - 30.00)	0.0001
Total	50	25.64 ± 5.17	(18 - 37)	(24.21 - 27.07)	33 (66.00%)	27.15 ± 4.75	(18 - 36)	(25.53 - 28.77)	0.0022

		UTM samples			***Guthrie cards***				
	N	$\bar{X} \pm \sigma$	*Range*	*IC95%*	*Sensitivity*	$\bar{X} \pm \sigma$	*Range*	*IC95%*	*p-value (0.05)*
log ≥ 5	37	7.28 ± 1.28	(5.26 - 11.12)	(6.87 - 7.69)	23 (62.16%)	6.66 ± 1.54	(3.95 - 9.31)	(6.16 - 7.16)	0.0241
4 ≤ log ≤ 4,99	7	4.51 ± 0.26	(4.16 - 4.99)	(4.31 - 4.71)	5 (71.43%)	6.87 ± 1.36	(5.44 - 9.02)	(5.87 - 7.87)	< 0.0001
log ≤ 3,99	6	3.54 ± 0.40	(2.92 - 3.96)	(3.22 - 3.86)	5 (83.33%)	5.98 ± 0.71	(5.15 - 6.93)	(5.41 - 6.55)	< 0.0001
Total	50	6.52 ± 1.86	(2.92 - 11.12)	(6.00 - 7.04)	33 (66.00%)	6.58 ± 1.41	(3.95 - 9.31)	(6.19 - 6.97)	0.7910