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Pooling of nasopharyngeal swabs to identify asymptomatic cases of SARS-CoV-2 virus during first wave of the COVID-19 pandemic

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RESUMEN

Agrupación de exudados nasofaríngeos para identificar casos asintomáticos de SARS-CoV-2 durante la primera ola pandémica de COVID-19.

Introducción. Los sistemas de salud del mundo han padecido dificultades durante la pandemia de COVID-19. Los portadores asintomáticos dificultaron la vigilancia y seguimiento de individuos infectados. Así mismo, los laboratorios de diagnóstico padecieron deficiencias en reactivos. Agrupar las muestras clínicas es una alternativa para sobrellevar la poca disponibilidad de reactivos y el diagnóstico de individuos asintomáticos.
Métodos. Se analizaron 1937 muestras clínicas de personas asintomáticas que realizaban actividades esenciales durante la primera ola de COVID-19 en Yucatán, México. Se realizó diagnóstico por RT-PCR en tiempo real en 229 agrupaciones de muestras.
Resultados. Se detectaron casos positivos de personas asintomáticas con COVID-19 en 27 agrupaciones. Los individuos positivos realizaban actividades esenciales en la administración del gobierno del estado, en supermercados o en la fuerza policiaca.
Conclusión. La agrupación de muestras clínicas es una estrategia para facilitar el aislamiento oportuno de individuos infectados asintomáticos, así como para ahorrar en reactivos de laboratorio.

ABSTRACT

Introduction. Public and private health services worldwide have faced difficulties under the COVID-19 pandemic. High numbers of asymptomatic carriers difficulted the surveillance and tracing of

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infectious individuals. Also, diagnostic laboratories suffered shortage of reagents to perform on-time diagnosis. Pooling of clinical samples offers an alternative to overcome reagents unavailability and diagnosis of asymptomatic individuals.

Methods. We performed pooling of 1937 clinical samples from asymptomatic people performing essential activities during the first wave of the COVID-19 pandemic in Yucatan, Mexico. Diagnosis by real time RT-PCR using the Berlin Method was performed in 229 pools.

Results. The strategy successfully detected asymptomatic COVID-19 positive cases. A total of 27 pools were positive. Individuals performing essential activities as government administrative, food markets and police force were the most commonly positive.

Conclusion. *Pooling* clinical samples is a strategy for the on-time isolation of infected individuals as well for saving laboratory resources.

INTRODUCTION

During the COVID-19 pandemic, the laboratory diagnosis of SARS-CoV-2 viral infection have been limited by continues supply shortages worldwide, hampering the testing capacity of laboratories. *Pooling* of clinical samples is a strategy to overcome those difficulties (1), and its efficacy has been demonstrated (2–5) an increase in workload and medical expenses have been a concern to the health care system worldwide. Developing a measure that helps to conserve the health care resource is, therefore, highly desirable, and the pooling of the specimens for testing is one of the attractive strategies. Recently, we showed that saliva could be a potential alternative specimen for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2. Since the early stages of the COVID-19 pandemic, governments worldwide implemented non-pharmaceutical interventions (NPIs) to reduce the spread of the coronavirus (e.g. partial or strict lockdown), however, numerous essential service providers such as pharmacists, food markets vendors, health professionals, government and municipal administrative personnel as well as police forces remained operating to satisfied population main needs.

We report the results of implementing a *pooling* strategy of naso-and-oropharyngeal swabs (NOS) to screen large number of samples from individuals with essential activities and at high risk of having asymptomatic infections during the first wave of the COVID-19 pandemic. The results of the laboratory screening helped the health authorities to implement highly effective early interventions, followed by enforced quarantines and isolations to disrupt transmission in this population.

METHODS

The Health Ministry of Yucatan, Mexico, implemented a strategy for tracing of asymptomatic transmission of COVID-19 from May 13th to June 30th, 2020. A pooled testing approach was performed in 1,937 NOS collected between May 29/2020 to June 26/2020 from individuals providing essential activities including health personnel (505), police force and/or security personnel (414), administrative personnel from government or the municipality offices (340), food market personnel (170), public transportation operators and taxi drivers (98), hospital janitors (86), and other essential activity providers (e.g. workers in a bottled water company) (324).

The pool size (approximately 10 samples/pool) was selected using the Pooled testing web-based application (https://bilder.shinyapp.io/PooledTesting/), considering a prevalence of COVID-19 of 0.001 until May 29, an assay sensitivity of 95%, a specificity of 99%, a two-stage pooling algorithm, and a range of pool size of 10 samples. These calculations predicted a pool size of 10 samples that would provide a reduction in the expected number of tests of 88% when compared to individual testing.

Pools and individual samples were analyzed by real time RT-PCR to amplify a segment of the envelope (*E*) gene and the constitutive ribonucleoprotein (RP) gene according to the Berlin method reported by the Charité Institute (6). Samples with threshold cycles (Ct) $\leq$ 38 were considered positive. Viral

RNA was extracted from pools of 10 individual clinical samples using the QIAamp Viral RNA Mini extraction kit (Qiagen). If the pool were positive, RNA extracted from individual samples was assessed afterwards using the same RT-PCR method described above.

The sensitivity of the pooling strategy to detect one or more positive samples in each pool, expressed as copies/µL, was confirmed using a standard curve. Ten pools of 10 samples with known Ct values (at least one positive vs negatives) were mixed using equal volumes (10 µL each) and processed for RNA extraction, followed by RT-PCR analyses. Ct values obtained using the standard curve were compared with the Ct values obtained from the samples tested in this study.

RESULTS.

Results from the standard curve (Figure 1a) showed that pooling of 10 clinical samples was able to detect positive samples with Ct values close to the limit of detection (Figure 1a). For instance, pools # 6 and 9 showed the highest Ct mean values: 36.46 and 36.39 which correspond to the lowest number of copies/µl: 39.81 and 41.59, respectively (Table 1). Compared to the original method, which limit of detection was 3.9 copies per reaction of the gene E (6), our method showed a limit of detection of 1 copy /µL (Figure 1a).

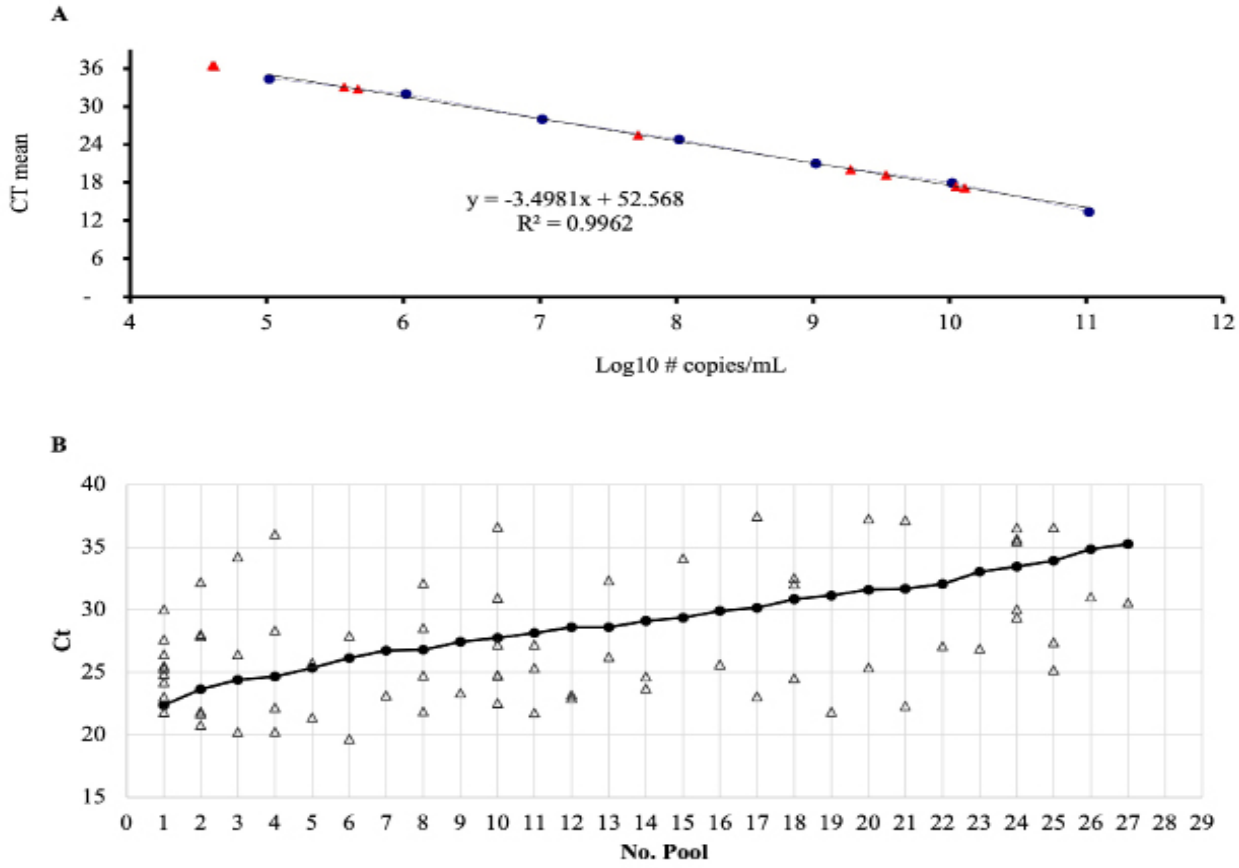

Figure 1a. Standard curve for ten pools with known Ct mean values in red triangles. Blue circles correspond to serial dilutions of pDrive plasmid containing the *E* gene (kindly donated by E. Ramírez-González, Unidad de Desarrollo Tecnológico, InDRE). Plasmid dilutions range from 1 to 1x10^6 ng/ml. Figure 1b. Cycle threshold (Ct) values for the gene *E* detected in the SARS-CoV-2 positive pools (n=27; filled circle) as well as in each individual sample that compose each pool (n=72; empty triangles).

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Table 1. Cycle threshold values of *E* gene in pools and individual samples analyzed in the standard curve*.

Pool	No. samples/*pool* (No. Positive)	*Pool* Ct Mean ± SD	Ct Individual Positive Samples *E* gene		
1	10	Negative	-	-	-
2	10(3)	17.41 ± 0.65	19.49	18.77	19.05
3	10(3)	19.2 ± 0.24	27.43	27.12	20.88
4	10(3)	33.07 ± 0.19	36.76	32.07	36.57
5	10(3)	20.11 ± 0.13	18.77	29.13	37.93
6	10(2)	36.46 ± 0.37	32.84	36.27	
7	10(1)	32.73 ± 0.13	30.95		
8	10(1)	17.19 ± 0.05	19.05		
9	10(1)	36.39 ± 2.4	36.79		
10	2(2)	25.54 ± 0.09	25.89	31.42	

* Standard curve was calculated with the formula Y=mx + b, where Y intercepts at 1 ng of RNA. The efficiency of the reaction was calculated with the formula Efficiency = [10^(-1/slope) - 1] x 100.

Overall, a total of 229 pools (ranging from 6 to 10 samples) were analyzed using this pooling approach showing a positivity of 11.8% (27/229). Regarding samples individually tested, 72 were positive with Ct values ranging between 19.66 to 37.46. Pools with one positive sample represented 33.3% (9/27) of all positive pools, two or more positive samples were detected in the remaining 18 pools (66.6%). Interesting, in one positive pool (pool No. 1), the individual analysis identified that all 10 samples were positive (Figure 1b). Positive samples were distributed in the following categories, health personnel (3), police force (8), government or municipal administrative personnel (12), food markets personnel (19), workers from a bottled water company (11), and cleaners (3). The remaining 16 samples were collected from prisoners and personnel from a readaptation center.

DISCUSSION

The results of our study demonstrate that the *pooling* testing strategy facilitate the process of larger number of samples in a short period of time compared to individual testing, which save reagents and supplies. An average of 100 clinical samples were daily analyzed, translated into only 10 pools/day. Thus, we saved a total of 5.7 extraction kits (250 columns each kit) and 2, 876 RT-PCR reactions. Furthermore, the significant value of our study was also to implement the diagnosis of asymptomatic people well-known to play a key role in virus transmission during the beginning of the pandemic, where timely decisions needed to be taken to break the chain of transmission.

The positivity of SARS-CoV-2 in Yucatan during the first wave of the COVID-19 pandemic was around 0.1 to 0.58 in symptomatic individuals, it remained similar during the months of May and June (7). However, during high transmission scenarios, the pooling strategy should not replace individual testing and the pool size should be choose relative to the prevalence (8). In conclusion, *pooling* testing represents a valuable strategy to expand the COVID-19 screening to cover more individuals, including asymptomatic people.

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Competing Interests

The authors declare noncompeting interests.

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