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*Original Article*

# Detection of SARS-CoV-2 by RT-qPCR in pooled samples of staff and patients at hospitals of Sonora, Mexico

Detección de SARS-CoV-2 por RT-qPCR en muestras agrupadas de personal y pacientes en hospitales de Sonora, México

**Liliana Carolina Rojo Arreola[1], Jorge Hernández López[2], Daniel Coronado Molina[2], Marcos José Serrato Félix[3], Joaquín Sánchez González[3] and Alvaro Santos Romo[2]***

[1] CONACYT-Centro de Investigaciones Biológicas del Noroeste S.C. Av. Instituto Politécnico Nacional 195, Col. Playa Palo de Santa Rita Sur; La Paz, B.C.S. México; C.P. 23096.
[2] Centro de Investigaciones Biológicas del Noroeste S.C. Hermosa No. 101 Col. Los Ángeles, Hermosillo, Son., México; CP. 83106.
[3] Hospital General del Estado "Dr. Ernesto Ramos Bours". Boulevard Luis Encinas Johnson S/N, Col. San Benito, Hermosillo, Son., México; C.P. 83000.

## ABSTRACT

The RT-qPCR method is highly reliable for detecting SARS-CoV-2, but its cost hinders widespread use in large-scale screenings. This study aimed to assess and validate cost-effective alternative protocols for mass testing in low-morbidity, high-risk environments. Fifty patient samples from the COVID in-patient care area and 50 samples from Hospital General del Estado de Sonora personnel were analyzed using a pooling approach in varying prevalence settings. Individual RNA was pooled in groups of five, then tested for SARS-CoV-2 via RT-qPCR. This pooling strategy was employed for diagnosing SARS-CoV-2 in medical personnel, involving 885 HGE staff samples and 100 from Hospital Infantil del Estado de Sonora during the peak of the pandemic. Significant reductions in the number of RT-qPCR reactions were observed: a 77 % decrease in analysis cost for the 885 samples from Hospital General del Estado de Sonora staff, and an 80 % reduction in reactions needed for the 100 samples from HIES staff. This study demonstrated the effectiveness and efficiency of analyzing pooled samples for widespread SARS-CoV-2 diagnosis in a population at low risk but with high exposure. This approach can be implemented in settings with high spatiotemporal density to mitigate hospital-based transmission risks.

**Keywords:** COVID-19; SARS-CoV-2; RNA Pool.

## RESUMEN

El método de RT-qPCR es altamente confiable para detectar el SARS-CoV-2, pero su costo dificulta su uso generalizado en cribados a gran escala. Este estudio tuvo como objetivo evaluar y validar protocolos alternativos rentables para pruebas masivas en entornos de baja morbilidad pero alto riesgo. Se analizaron 50 muestras de pacientes del área de cuidados intensivos de COVID y 50 muestras del personal del HGE utilizando un enfoque de agrupación en diferentes configuraciones de prevalencia. El ARN individual se agrupó en grupos de cinco, luego se probó para SARS-CoV-2 mediante RT-qPCR. Esta estrategia de agrupación se utilizó para diagnosticar SARS-CoV-2 en personal médico, involucrando 885 muestras de personal del HGE y 100 del HIES durante el pico de la pandemia. Se observaron reducciones significativas en el número de reacciones de RT-qPCR: una disminución del 77 % en el costo de análisis para las 885 muestras del personal del HGE, y una reducción del 80 % en las reacciones necesarias para las 100 muestras del personal del HIES. Este estudio demostró la efectividad y eficiencia del análisis de muestras agrupadas para el diagnóstico generalizado de SARS-CoV-2 en población de bajo riesgo pero con alta exposición. Este enfoque puede implementarse en entornos con alta densidad espaciotemporal para mitigar los riesgos de transmisión hospitalaria.

**Palabras clave:** COVID-19; SARS-CoV-2; Pool ARN.

## INTRODUCTION

The current epidemic caused by the SARS-CoV-2 virus, not only in Mexico but throughout the world, has seriously affected the health of communities and has generated an economic crisis that jeopardizes the stability of many countries (Deka *et al*., 2020). Given that SARS-CoV-2 transmission is airborne and viruses can travel attached to airborne particles (Hernández *et al*., 2021), surveillance of hospital personnel (patients and medical staff), as well as individuals frequenting public spaces such as airports and schools, in addition to contact tracing of close contacts of COVID-19 patients, should be prioritized in the Ministry of Health's surveillance programs. Early detection of the pathogen significantly contributes to reducing the infection rate, particularly in confined environments such as hospitals and offices. Epidemiological surveillance, contact tracing, and analysis of asymptomatic individuals are fundamental components in the fight against the spread of SARS-CoV-2. These strategies allow for early case detection, rapid identification of outbreaks, and timely implementation of control measures, which is crucial for mitigating virus transmission in the community. Furthermore, the analysis of asymptomatic individuals is particularly relevant, as they can act as silent carriers, significantly contributing to the unknowingly spread of the disease. The effective im-

*Author for correspondence: Alvaro Santos Romo
e-mail: , asantoso4@cibnor.mx
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plementation of these measures not only helps contain the pandemic but also provides valuable data to inform public health policies and optimize response strategies for future health threats.

To this date, the RT-qPCR technique is the most reliable for the detection of SARS-CoV-2. However, its cost is high compared to other available methods, making it difficult to use in large-scale population screening. Although SARS-CoV-2 detection methods have evolved to be significantly faster and more cost-effective, there is a need to test large numbers of individuals to increase epidemiological surveillance and to reduce the transmissibility of the virus, which is still affected by the high cost of analysis (Deka *et al*., 2020; Garg *et al*., 2021). Furthermore, a global shortage of supplies for RNA extraction and detection has been reported, which is mainly driven by an increase in demand, a problem that is more severe during outbreaks (Deka and Kalita, 2020; Farfan *et al*., 2020; Yelin *et al*., 2020). A low-cost strategy confers an advantage in increasing the number of analyzed samples and therefore the possibility of preventing the spread of the virus in susceptible communities. These facts are relevant for middle-income countries such as Mexico, where large-scale detection is not feasible.

Pooling samples for SARS-CoV-2 detection is a widely used method that allows prompt analysis of many samples at a lower cost and with a significant reduction in supplies. Pooling procedures vary from mixing several nasal swab specimens (Chen *et al*., 2020; Chong *et al*., 2020; Farfan *et al*., 2020; Garg *et al*., 2021; Yelin *et al*., 2020) or saliva samples (Barat *et al*., 2021), to mixing individual samples of extracted RNA (Deka *et al*., 2020; Deka and Kalita, 2020; Gupta *et al*., 2020; Mulu *et al*., 2021; Pasomsub *et al*., 2021) for a single analysis with RT-qPCR.

There are several reports on the use of pooled samples in the detection of SARS-CoV-2 using molecular techniques (Cabrera *et al*., 2020; Chen *et al*., 2020; Chong *et al*., 2020; Deka *et al*., 2020; Farfan *et al*., 2020; Gupta *et al*., 2020), a general conclusion is that this approach is particularly convenient in low-prevalence areas or in population sectors where massive epidemiological surveillance is required (Ben-Ami *et al*., 2020; Chen *et al*., 2020; Deka *et al*., 2020; Garg *et al*., 2021), This strategy can be used for early SARS-CoV-2 detection and can reduce the risk of transmission for company workers, health care staff, students, etc., but the adequacy of this strategy should be determined by each laboratory (Vitro Master Diagnóstica, 2021).

In this work, the implementation of a pooling strategy for detection of SARS-CoV-2 in RNA extracted from nasal specimens was developed, this approach has been widely proposed **(**De Salazar *et al***.,** 2020**;** Hogan *et al***.,** 2020**;** Lagopati *et al***.,** 2021**;** Mulu *et al***.,** 2021**;** Mutesa *et al***.,** 2021**)** but only a few experimental demonstrations exist in Mexico (Herrera *et al*., 2021). The presence of SARS-CoV-2 was screened in staff and hospitalized patients at the HGE (Hospital General del Estado) in Hermosillo, Sonora, México. RT-qPCR tests were conducted using pooled RNA samples as a template, with the aim of evaluating and validating the performance of alternative protocols that are cost-effective for mass testing of SARS-CoV-2 in low-morbidity, high-risk settings.

## MATERIAL AND METHODS

This study was reviewed and approved by the Research Ethics Committee of the Hospital General del Estado Dr. Ernesto Ramos Bours. The research protocol, titled "Technical and scientific contribution to strengthen the diagnostic capacity of the Health Sector in Sonora for COVID-19 detection", was evaluated and received ethical approval with the registration number CEI 2022-37. The approval was granted on May 4, 2022, following the ethical procedures and standards established by the committee.

Specimens were collected by trained medical personnel at HGE and analyzed at Laboratorio de Referencia, Análisis y Diagnóstico en Sanidad Acuícola (LARADSA) attached to Centro de Investigaciones Biológicas del Noroeste (CIBNOR)-Hermosillo, Sonora, México, which is authorized by the Mexican jurisdiction of epidemiology (Instituto de Diagnóstico y Referencia Epidemiológicos–InDRE), for handling and processing samples for SARS-CoV-2 analysis.

To test the efficiency and sensitivity of pooling individual RNA samples for SARS-CoV-2 detection using RT-qPCR, 63 negative samples stored 30 d at -80 °C from our repository were randomly selected. Random SARS-CoV-2 positive RNA samples were used to prepare pools at different ratios. Pools of five individual RNAs containing cero, one, two and five positive RNAs were randomly mixed and tested using RT-qPCR. In addition, pools of six and ten individual RNAs containing only one positive sample were tested. Specimens with a CT value (number of cycles in which the fluorescent signal crosses the threshold) below 35 were deemed positive.

Nasopharyngeal and oropharyngeal exudates were obtained using a dacron and cotton swab, respectively, and submerged in a 15 mL tube that contained 3 mL of transport solution (DNA/RNA Shield™) that inactivated the virus and preserved the RNA in the samples. Consent forms were provided for each specimen. The samples were preserved and transported at 4 °C to LARADSA and processed for SARS-CoV-2 testing.

RNA was individually isolated from 200 µL of each specimen using the Quick-RNA Viral kit (ZymoResearch), following the manufacturer's instructions.

After extraction, 10 µL of each specimen were used to form pools of five patients, and the samples were mixed in a nuclease-free tube to obtain a final volume of 50 µL. This RNA pooling approach allows individual samples to be tested in pools that are positive for SARS-CoV-2, eliminating the need for a new RNA extraction method.

The RT-qPCR reaction was performed using the commercial kit SARS-CoV-2 RT-qPCR from VITRO, following the manufacturer's instructions. Eight microliters of each pooled RNA sample were used as the template. This kit is a multiplex system that amplifies a gene fragment of the N and E proteins from SARS-CoV-2 and a gene fragment of RNaseP

from humans. This kit is approved by the Mexican regulatory authority for medical devices (COFEPRIS and InDRE) and can detect the currently dominant omicron SARS-CoV-2 variant according to in silico analysis (Vitro Master Diagnóstica, 2021).

All reactions were run in a real-time thermal cycler CTX96 (Bio-Rad), and the reaction profile consisted of 15 min at 50 °C, followed by incubation for 2 min at 95 °C. Finally, 40 cycles of 15 s at 95 °C and 30 s at 58 °C were performed. Figure 1 presents a diagram illustrating the process of the implemented methodology.

A quantitative approach was employed to analyze the prevalence of SARS-CoV-2 among healthcare workers. Chi-square tests with a 95 % confidence level were conducted to assess associations between categorical variables. Specifically, the relationship between the number of SARS-CoV-2 positive workers and the work area was examined, as well as the association between infection and workers' sex. Additionally, SARS-CoV-2 prevalence percentages were calculated, stratified by age groups and sex.

The suitability of the five-sample pooling approach in a real case scenario was tested in two different settings: samples obtained from patients in the COVID inpatient area at HGE (n = 50, high-prevalence scenario) and samples obtained from medical staff at HGE (n = 50, low-prevalence scenario). Finally, an epidemiological description was conducted using the five-specimen pooling approach on 885 and 100 healthcare staff samples at HGE and Hospital Infantil del Estado de Sonora (HIES), respectively.

## RESULTS AND DISCUSSION

Several reports have determined that a pool of five specimens is effective without compromising the sensitivity of PCR detection of SARS-CoV-2 (Abdalhamid *et al*., 2020; Alcoba *et al*., 2021), therefore, an initial test was conducted to prove that analyzing groups of five specimens is a viable way to detect the presence of SARS-CoV-2, even if only one sample is positive. Amplification in all pools containing one, three, or five positive samples was observed.

As expected, the CT values of pooled specimens tended to decrease as the number of positive samples in the pool was increased. However, the sensitivity observed was not compromised in the five-sample pools. The pool with a high samples number could be severely affected as the number of samples increase.

Although pool of ten specimens containing only one positive sample was acceptably amplified by RT-qPCR, the CT value was affected in an important way (Figure 2). Due to this, five samples were used in each analyzed group.

The versatility of the five-specimen pooling method of low and high prevalence scenarios was tested. In both scenarios, 50 patients samples from the COVID area and 50 samples from HGE medical personnel were analyzed. For high-prevalence populations, 70 % of the analyzed pools tested positive. When individual specimens from positive pools were analyzed, a prevalence rate of 26 % was found. For this scenario, 45 RT-qPCR reactions were required to diagnose the 50 samples, indicating a reduction of only 10 % of the total PCR reactions.

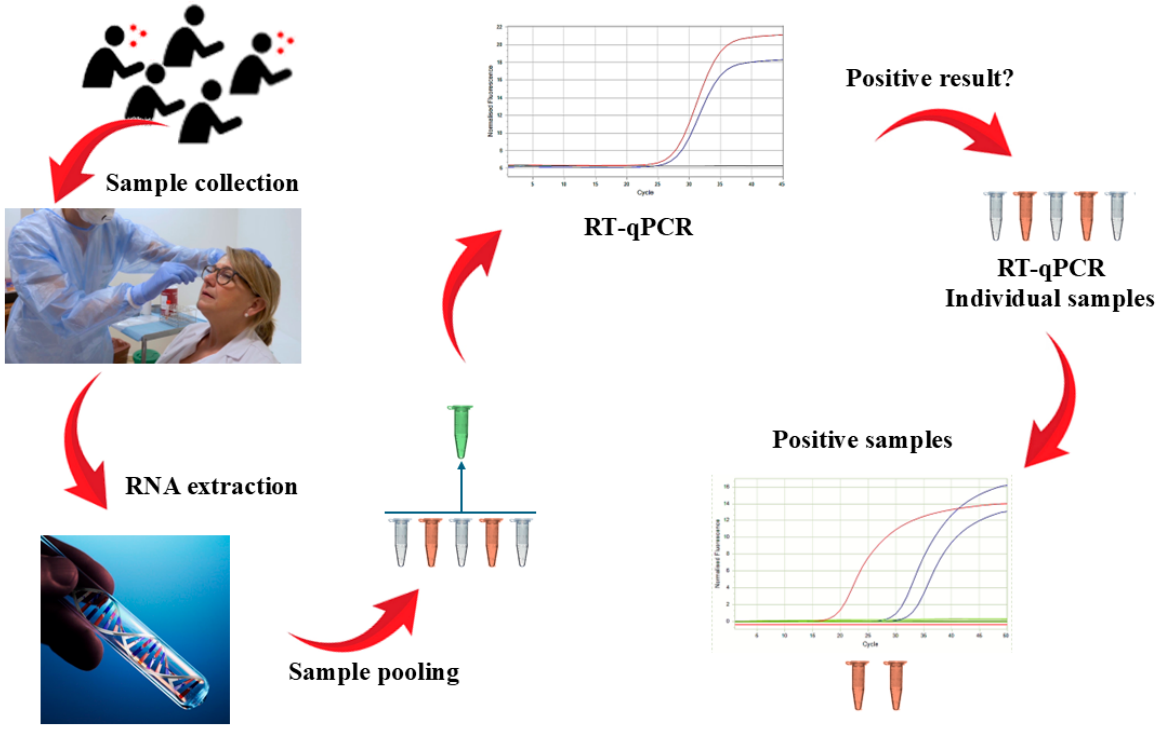


Figure 1. Diagram of the implemented methodology.
Figura 1. Diagrama de la metodología implementada.

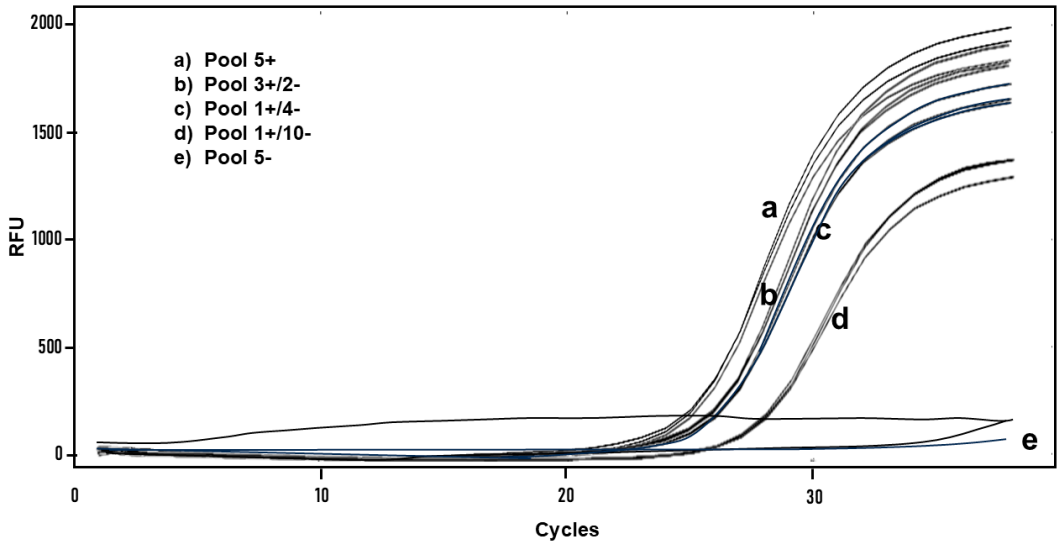


**Figure 2.** RT-qPCR of RNA analyzed in pools of 5 where samples 1, 3 and 5 are positive for SARS-CoV-2 (a, b, c) and samples in pools of 10 containing 1 positive (d).
**Figura 2.** RT-qPCR de ARN analizado en grupos de 5, donde las muestras 1, 3 y 5 son positivas para SARS-CoV-2 (a, b, c), y muestras en grupos de 10 que contienen 1 positivo (d).

For the low prevalence scenario, 50 samples of HGE healthcare personnel were analyzed. Ten reactions were required for the analysis of 50 specimens, which represented an 80 % reduction in the number of PCR reactions; a prevalence rate of 0 % was observed (Table 1).

A description of the epidemiological features of SARS-CoV-2 positive samples from medical personnel tested using the pooled sample approach was provided. RT-qPCR was performed on 885 and 100 samples from HGE and HIES staff, respectively. The number of reactions required to analyze all specimens was 202 for HGE and 20 for HIES (Table 2). This strategy significantly reduced the amount of required materials, resulting in savings of 77 % (HGE) and 80 % (HIES) in project costs.

Analysis of the results revealed a low prevalence of SARS-CoV-2 infection (1.36 %). Among the SARS-CoV-2 positive individuals, 43 % were in the age range of 21–30 years, while 57 % were in the age range of 51 – 60 years (Figure 3).

**Table 1.** Analysis of 50 COVID patients at the COVID inpatient unit and 50 samples of health care workers at HGE. The samples were analyzed in groups of five. The positive pools were individually evaluated to detect the total number of positive patients.
**Tabla 1.** Análisis de 50 pacientes con COVID en la unidad de pacientes hospitalizados con COVID y 50 muestras de trabajadores de la salud en el HGE. Las muestras fueron analizadas en grupos de cinco. Los grupos positivos fueron evaluados individualmente para detectar el número total de pacientes positivos.

| | Samples from patients | | | Samples from health care workers | | |
|---|---|---|---|---|---|---|
| Pool ID | Result | Individual sample positive | Number of RT-qPCR reactions | Result | Individual sample positive | Number of RT-qPCR reactions |
| 1 | + | 2 | 6 | - | 0 | 1 |
| 2 | + | 2 | 6 | - | 0 | 1 |
| 3 | + | 1 | 6 | - | 0 | 1 |
| 4 | - | 0 | 1 | - | 0 | 1 |
| 5 | + | 1 | 6 | - | 0 | 1 |
| 6 | + | 4 | 6 | - | 0 | 1 |
| 7 | - | 0 | 1 | - | 0 | 1 |
| 8 | - | 0 | 1 | - | 0 | 1 |
| 9 | + | 1 | 6 | - | 0 | 1 |
| 10 | + | 2 | 6 | - | 0 | 1 |
| TOTAL TESTS | | 13 | 45 | | 0 | 10 |

(+) Positive, (-) Negative

**Table 2.** Analysis of 885 samples from medical staff at HGE and 100 at HIES. The samples were analyzed by pooling five specimens. The positive pools were individually analyzed to detect the prevalence of SARS-CoV-2.
**Tabla 2.** Análisis de 885 muestras del personal médico en el HGE y 100 en el HIES. Las muestras fueron analizadas agrupando cinco especímenes. Los grupos positivos fueron analizados individualmente para detectar la prevalencia de SARS-CoV-2.

| | HGE | HIES |
|---|---|---|
| Samples analyzed | 885 | 100 |
| Positive pools | 5 | 0 |
| Negative pools | 172 | 20 |
| Initial RT-qPCR reactions | 177 | 20 |
| Individual RT-qPCR reactions | 25 | 0 |
| Total RT-qPCR reactions | 202 | 20 |

The analysis of SARS-CoV-2 infection related to sex detected a higher prevalence in women (0.9 %) than in men (0.45 %), but a chi-square test showed no difference between these two groups. The average age of the SARS-CoV-2 positive personnel at HGE was 32 years.

A chi-squared test with a 95 % confidence level was used to assess the association between the number of workers who tested positive for SARS-CoV-2 and work area. A significant relationship between healthcare staff and positivity rate was found, indicating that the staff in direct contact with patients is at a higher risk of infection with SARS-CoV-2, compared to laboratory ($\lambda^2 = 0.0085$: $P < 0.05$) and administrative ($\lambda^2 = 1.71$: $P < 0.05$) staff (Figure 4).

Many studies have proven that asymptomatic COVID-19 carriers are major contributors to virus propagation (Bai *et al*., 2020; Gao *et al*., 2021; Nishiura *et al*., 2020; Schuetz *et al*., 2020). One of the best strategies to reduce infections by healthy carriers is epidemiological surveillance, contact tracing, and analyzing asymptomatic individuals in addition to patients with symptoms. However, the surveillance of asymptomatic individuals can be challenging, especially in settings with limited resources, due to the high cost, not only of testing supplies but also of human resources, because it implies conducting a community risk assessment.

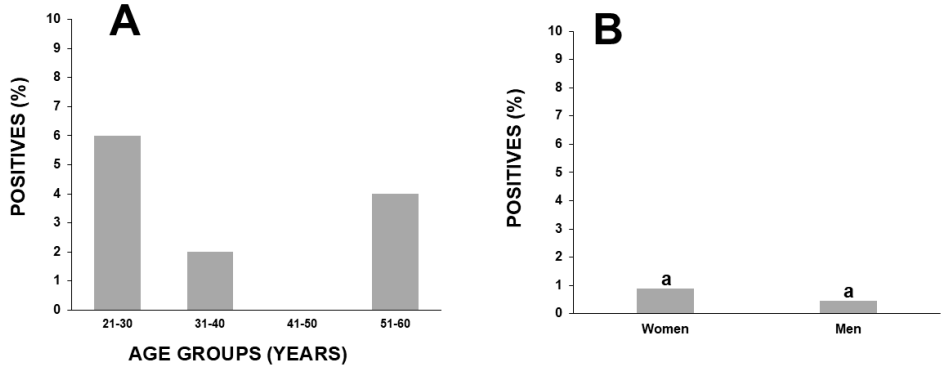


**Figure 3.** A) The age range with higher prevalence was from 21 to 30 years. B) It was observed higher prevalence in women than in men but no differences was demonstrated by Chi squarer test.
**Figura 3.** A) El rango de edad con mayor prevalencia fue de 21 a 30 años. B) Se observó una mayor prevalencia en mujeres que en hombres, pero no se demostraron diferencias significativas mediante la prueba de Chi-cuadrado.


**Figure 4.** Total SARS-CoV-2 positive workers at HGE analyzed by the work area. At the time of the study, all administrative workers tested negative for SARS-CoV-2.
**Figura 4.** Total de trabajadores positivos para SARS-CoV-2 en el HGE analizados por área de trabajo. En el momento del estudio, todos los trabajadores administrativos dieron negativo para SARS-CoV-2.

The massive analysis of individuals using pooled samples is a promising strategy for effectively screening a large population, reducing the cost of the tests and improving the response time, aiding in minimizing the chance of spreading the disease in a population as a preventive measure of viral infection (García *et al*., 1996; Roth *et al*., 1999). Pool testing for COVID-19 has received emergency use authorization from the U.S. Food and Drug Administration in July 2020, with the premise that each laboratory must determine the feasibility of this approach, since the conditions for nucleic acid extraction and amplification vary for each case. The pooling method and effective pool size should be experimentally investigated.

Three strategies for pooling samples in the detection of SARS-CoV-2 have been reported: a) pooling swabs in a single vial, b) mixing samples from different specimens before RNA extraction and c) mixing RNA extracted from individual patients. Strategies b and c are the most recommended and both methods have been shown to be equally sensitive, although in some cases RNA pooling turned out to be more sensible and even more cost-effective (Mulu *et al*., 2021). For this study, the RNA sample pooling strategy was used.

In sample pooling for SARS-CoV-2 detection, a key parameter is determining the optimal pool size. Reported numbers vary widely, ranging from five to 30 samples per pool (Deka *et al*., 2020), three to ten (Abdalhamid *et al*., 2020), six to ten (Chen *et al*., 2020), six to 11 (Deka and Kalita, 2020) or three to 64 (Yelin *et al*., 2020). A pool of fewer than ten samples is widely recommended since the loss of sensitivity is within an acceptable threshold. Since the sensitivity improves as the sample number in the pool decreases, a pool size of four or five samples is generally reported as optimal (Abdalhamid *et al*., 2020; Alcoba *et al*., 2021). When evaluating the differences in CT values when pooling five samples, including one, three, and five positives, an increase of 1.0 in CT value was observed in the group with only one positive sample. However, when pooling ten with a single positive sample, a significant increase in the CT value was observed, therefore, after considering a balance between sensitivity and saving resources, the pool of five samples was selected.

The pooling technique for SARS-CoV-2 detection has been widely utilized in various parts of the world (Ben-Ami *et al*., 2020; Chong *et al*., 2020; Deka *et al*., 2020; Deka and Kalita, 2020; Lagopati *et al*., 2021; Millioni and Mortarino, 2020; Yelin *et al*., 2020), as a form of a high-throughput SARS-CoV-2 diagnostic tool in low-prevalence communities with a significant decrease in resources; however, to date, there have been no reports on the implementation of active surveillance of SARS-CoV-2 in Mexico. In our laboratory, the daily maximum number of individual PCR reactions that can be processed is 180, using the five samples pooling approach, we can process up to 900 samples, which significantly improves our response capacity and reduces the turnaround time. This strategy greatly improves patient care and prompt decision-making. A system was implemented in which the sample processing, molecular tests, and report of results were performed in five to six h, depending on the number of samples.

A probabilistic model of sample pooling strategies for COVID-19 testing demonstrated that the approach is advantageous only if the prevalence is lower than 30 %, beyond this percentage is no longer cost-effective (Cherif *et al*., 2020). Two scenarios were analyzed, in the high prevalence setting (26 %), only 10 % fewer PCR reactions were needed to analyze all samples, demonstrating that the strategy of grouping samples in populations with comorbidities is not recommended since to the reduction in the number of tests is not significant.

The five samples pooling approach allowed for massive analysis in susceptible communities. A significant reduction in supplies (77 % or 80 %) was observed, since to analyze two sets of healthcare personnel, 885 and 100 samples in each set, required only 202 and 20 RT-qPCR reactions, respectively.

Healthcare personnel are at high risk of SARS-CoV-2 infection by being exposed to potentially infectious patients in hospitals, and can also be a source of transmission by introducing a virus to the hospital, highlighting the importance of maintaining surveillance of the presence of this virus in all staff, both healthcare and administrative personnel (Velhal *et al*., 2022). In the present study, it was demonstrated that the implementation of low-cost active surveillance of SARS-CoV-2 in hospital centers is feasible, owing to the use of pooled samples for the analysis. This strategy can be transferred and implemented in other workplaces, such as companies, government offices, and schools.

The low incidence of SARS-CoV-2 infection observed among personnel working at HGE, indicates that most personnel follow the biosecurity measures implemented in the work area. Although the analysis of the samples from the personnel showed that there was no influence of sex or age on the infection, there was a significantly higher risk in the personnel who directly cared for patients with COVID-19.

The use of pooled samples for the analysis of SARS-CoV-2 may have some disadvantages, the main one being the decreased in sensitivity. However, the savings in time and resources due to the decrease in the necessary inputs are significantly lower; therefore, in this work, the grouping of a low number of samples, in comparison whit other approaches proposed in the literature, was considered.

## CONCLUSIONS

The results obtained in this work, showed the viability of pool testing for massive analysis in low-prevalence populations, which allowed the optimization of the testing capacity, enabling a significant increase in the number of individuals analyzed with an improvement in the levels of surveillance and prevention of SARS-CoV-2 infection, without compromising the efficacy of detection. This strategy can be used to screen large numbers of individuals in businesses, government offices, schools, etc., to encourage a safe return to productive activities. Additionally, the results obtained in this study verified that the measures implemented in the HGE are effective in maintaining the biosecurity required for the best care of patients requiring hospitalization.

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## CONFLICTS OF INTEREST

The authors report having no potential conflicts of interest.

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