Letter to the Editor

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Pre-enriched saline gargle samples for detection of SARS-CoV-2

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SUMMARY A self-collected gargle sample, which avoids discomfort and largely reduces the dependency on medical resources, is emerging for detection of SARS-CoV-2. However, the incomplete usage of starting materials for both routine oropharyngeal swabs (OPS)/nasopharyngeal swabs (NPS) and saline gargle (SG) samples implies sensitivity can be further improved. Presented here is a bead-based strategy for pre-enrichment of SG samples, and results revealed that it acquired about 20 times the starting materials obtained from OPS samples for downstream detection of SARS-CoV-2. The sensitivity and specificity of this pre-enrichment strategy were validated in 100 paired pre-enriched saline gargle (PenSG) and OPS samples and 89 PenSG samples from healthy volunteers. In addition to detection of SARS-CoV-2, this pre-enrichment strategy may also be implemented in more clinical settings to optimize detection of other diseases.

Keywords detection of SARS-CoV-2, saline gargle, pre-enrichment strategy, COVID-19

To the Editor,

Over the past three years, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), has infected more than 650 million, around 1/12 of the planet's population, and its variants are still evolving. Although the emerging variants, such as Omicron, have been found to cause less severe disease, they are much more infectious than the previous variants (*1*) and they continue to cause uncertainty worldwide. The shifting of public health policies in countries such as China and the newly acquired characteristics of SARS-CoV-2 variants require more timely, sensitive, and less clinically intensive testing strategies. Self-sampling and self-testing are two promising solutions. Antibody-based self-testing is easy to use, but it has relatively low sensitivity, especially for the dominant asymptomatic individual (*2*). Theoretically, sensitivity could be enhanced with SARS-CoV-2 nucleic acid self-testing, but large-scale population-based studies are needed to indicate its robustness. Moreover, such tests are not readily available in many countries and are relatively more expensive than other tests. Self-sampling coupled with a quantitative reverse transcription PCR (RT-qPCR) test represents a feasible and easily implemented approach at the current point in time.

A suitable method of self-sampling should comply with the following five criteria: 1) easily accessible/acquired by the public; 2) low dependency on medical resources; 3) user-friendly for most populations; 4) easy to standardize, thus ensuring the consistency of test results; and 5) compatible with downstream testing (Table 1). Saliva and gargle samples are emerging for detection of SARS-CoV-2 (*3*-*5*). Compared to conventional oropharyngeal swabs (OPS) and nasopharyngeal swabs (NPS), both saliva and gargle samples, which can be self-collected, avoid discomfort and largely reduce the dependency on medical resources. However, Landry *et al*. reported that pure saliva had a relatively low sensitivity (85.7%) in detecting SARS-CoV-2 (*5*), suggesting its unsuitability for high-volume testing and the need to optimize saliva collection and processing. Moreover, a study involving 108 patients with COVID-19 found that 46.3% experienced dry mouth (*6*), which in turn affects saliva production. Ease of standardization and sample accessibility are marked advantages of gargling samples over saliva. Recently, Qiao *et al* indicated that the saline gargle (SG) sample is also capable of detecting the currently dominant Omicron variants in both asymptomatic and symptomatic groups (*4*).

Currently, OPS and NPS are stored in 2-6 mL of virus preservation solution, and only 200 μL of sample is used for RNA extraction. In the aforementioned study, Qiao *et al*. also used 200 μL of saline gargle as the starting material for RNA purification and downstream detection of SARS-CoV-2 (*4*). All of these methods discard most of the materials. The incomplete usage of a

Table 1. The five basic principles of sampling to detect SARS-CoV-2

Strategy	Self-sampling		Non-self-sampling	
Type of sample	Gargle	Saliva	Oropharyngeal swab	Nasopharyngeal swab
Accessibility	Very easy	Limited in some situations, such as a dry mouth (*6*)	Difficult for individuals who are sensitive to sampling	
Dependency	No	Low	High	High
User-friendly	Yes	Yes	Causes slight discomfort	Cause moderate discomfort
Standardized	Easy	Hard	Depends on the skills and experience of medical personnel	
Compatibility	Yes	Yes	Yes	Yes

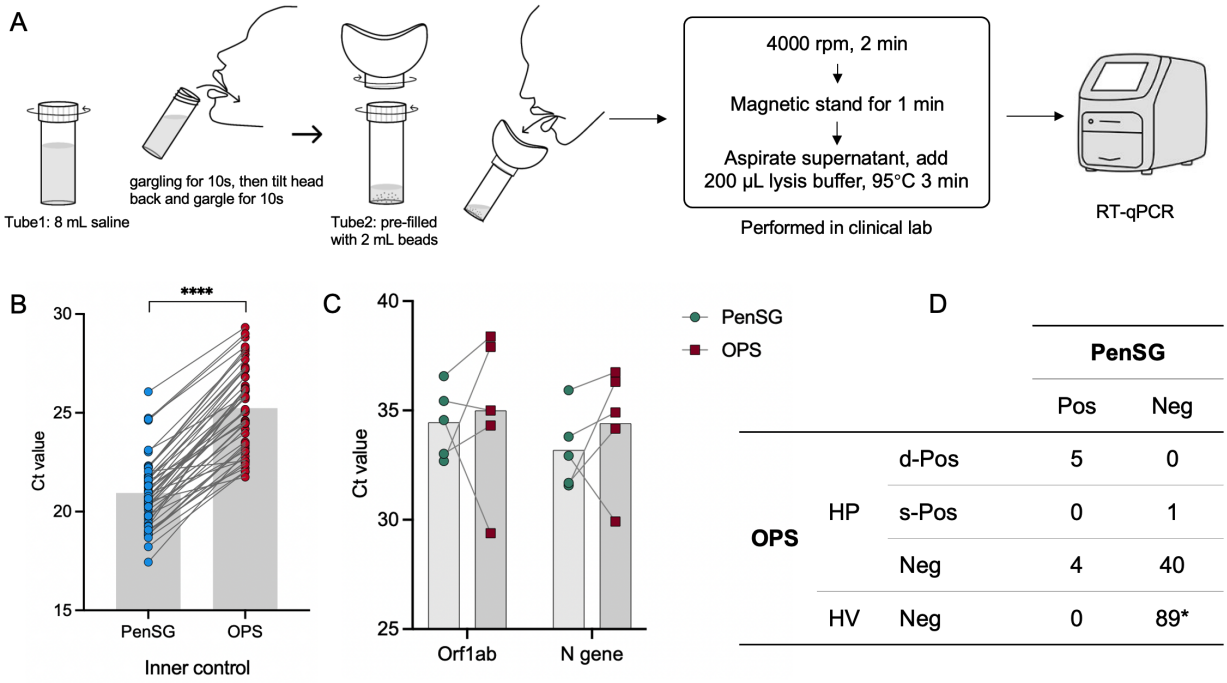

Figure 1. Using a pre-enriched saline gargle (PenSG) to detect SARS-CoV-2. (A) The steps in PenSG-based detection of SARS-CoV-2. **(B)** Ct values for the internal control in paired PenSG and OPS samples; **(C)** Ct values for the Orf1ab and N genes in paired PenSG and OPS samples in the group positive for both genes; **(D)** The overall performance of PenSG and OPS. 89* means the healthy volunteers tested negative but not at the same time with PenSG. HP, hospitalized patients; HV, healthy volunteers; d-pos, double-gene-positive; s-pos, single-gene-positive. In **(B)**, a paired *t*-test was conducted with GraphPad Prism 9; ****, *p*-value < 0.0001.

sample implies that the sensitivity of current methods can be further improved. To fully utilize all of a sample and to improve the sensitivity to the utmost extent, we pre-enriched SG samples with specialized beads that were conjugated to Concanavalin A (ConA) and able to bind to cells. Then, we use a magnetic stand to concentrate the beads and aspirate the supernatant of the saline gargle. Finally, a lysis buffer was directly added to the bead-bound system and the mixture was then subjected to routine RT-qPCR (Figure 1A).

To test the performance of this bead-based pre-enriched saline gargle (PenSG) in clinical settings, 100 paired PenSG and OPS samples were collected from 50 hospitalized patients with COVID-19 who were infected with the currently predominant SARS-CoV-2 Omicron variant (in different courses) in the Third People's Hospital of Shenzhen (June to July 2022). An additional 89 PenSG samples were collected from 89 healthy volunteers. This study strictly conforms to the provisions of the Declaration of Helsinki of the World Medical Association (2000) and was approved by the ethics committee of the Third People's Hospital of Shenzhen (no.2022-116-03). Detailed procedures and the clinical design can be found in the Supplementary Materials. All paired testing results are available in Table S1 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=131*).

First, the internal control (IC) for each paired sample was compared. It revealed that the cycle threshold (Ct) values for each PenSG were significantly lower than those for the OPS, with a mean differential Ct of 4.3 (20.95 *vs.* 25.24, Figure 1B), suggesting that PenSG could acquire about 20 (2^4.3) times the starting materials for downstream testing. Importantly, PenSG-based testing was able to detect all 5 patients who tested positive for both genes by the current routine strategy (OPS coupled with RT-qPCR). Most PenSG samples (3/5) had lower Ct values than OPS (Figure 1C), further

corroborating PenSG's superior sensitivity over OPS. Interestingly, PenSG detected 40 negatives among 44 cases detected negative for both genes by using OPS; the other 2 samples tested positive for a single gene and 2 samples tested positive for both genes. Since these samples came from hospitalized patients with COVID-19, special attention should be paid to these "false positives" and PenSG may help to improve the sensitivity of OPS to avoid potential "false negatives", thus establishing more strict decontamination criteria. Eighty-nine PenSG samples from healthy volunteers were used to further examine the specificity of PenSG, and all of the them were tested negative, further corroborating the specificity of PenSG. Taken together, these results validated the ability of PenSG to detect SARS-CoV-2 (Figure 1D).

SARS-CoV-2 enters the human body through the upper respiratory tract (URT); its viral load is associated with the risk of transmission, duration of infectiousness, disease severity, and mortality (*7*). PenSG, which is virus characteristic-based sampling, has all of the advantages of saline gargling but it also greatly enhances sensitivity by utilizing the whole sample, making it a robust alternative for detection of SARS-CoV-2. A limitation of the current study is mainly the relatively small sample size, precluding accurate assessment of the sensitivity and specificity of PenSG. However, this novel strategy revealed a long-neglected aspect of detecting SARS-CoV-2.

The biggest difference between the PenSG strategy and that used in the study by Qiao *et al*. is that PenSG utilized all 8 mL of the collected saline gargle to yield 200 uL of a nucleic acid solution without RNA purification, while the method used by Qiao *et al*. only used 200 uL from 8 mL of the collected saline gargle for RNA purification to yield 50 uL of nucleic acid eluate (according to the product manual) (*4*). The study by Qiao *et al*. revealed the advantage of SG over OPS as evinced by lower Ct values in the asymptomatic subgroup. However, PenSG can acquire about 20 times the materials as an OPS sample from all patients (regardless of symptoms and courses), suggesting that the unique PenSG strategy itself could further enhance sensitivity in addition to its advantage in terms of the type of sample. What makes PenSG stands out over previous approaches using a saline gargle and conventional OPS are that 1) PenSG can obtain more materials for downstream detection, thus further enhancing sensitivity; and 2) it avoids the RNA purification step, making this strategy especially suitable for in vitro diagnostics (IVDs).

A point worth noting is that the successful implementation of PenSG is based on these essential details: 1) the saline gargle can be standardized; 2) the efficiency of customized beads; 3) the lysis buffer, which is directly added to the beads without additional nucleic acid extraction steps, must be compatible with routine RT-qPCR kits. Fortunately, both the beads and lysis buffer are commercially available. In the near future, we are expanding the usage of PenSG to the detection of other viruses that infect the URT, such as the influenza virus and adenoviruses (*8*), and we will deploy this strategy of pre-enrichment in more settings, particularly for the detection of various diseases based on bodily fluids such as blood, urine, and even bronchoalveolar lavage fluid and vaginal secretions, in order to benefit a wider population.

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Conflict of Interest: Peng Xu and Wenqiang Yu are listed as inventors on pending patents related to this work; Jing Chen and Chengchen Qian are employees of Shanghai Epiprobe Biotech. Peng Xu used to be an employee of Shanghai Epiprobe Biotech. Wenqiang Yu serves on the Scientific Advisory Board of Shanghai Epiprobe Biotech.

Author Contribution Statements: Wenqiang Yu and Peng Xu conceived the project. Peng Xu developed the method under the supervision of Wenqiang Yu. Jing Chen and Chengchen Qian performed some of the experiments. Peng Xu drafted the manuscript. All authors read and approved the final version of the manuscript.

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