Objective: The objective of this study was to evaluate virucidal efficacy of XYNTRUS SMELL TEST MOUTHWASH against SARS-CoV-2 from nasopharyngeal samples.

Methodology:

Virus samples and products

SARS-CoV-2 positive nasopharyngeal swabs measured by RT-PCR with virus concentration between 6-8 Log10 copies/mL were kept at -80ºC. The solutions tested for virucidal activity in this study were: Iodopovidone (4mg); D-Limonene (0.3%); Cetylpyridinium Chloride 0.1% (CPC); Chlorhexidine gluconate 10% (CHX); Commercial mouth washes including PERIO-AID®; LISTERINE® ZERO ALCOHOL; SCOPE®; COLGATE® Plax, COLGATE® Periogard, CLORHEXOL® and XYNTRUS®. As a control reaction we used either saline solution or an excipient solution (water, glycerin, citric acid, colorant, sodium citrate).

Virucidal activity assay

Virucidal activity was measured by combining a previously quantified 1 part of high viral load nasopharyngeal sample with 1 part of the product or control (saline solution) for 1 minute with shaking. Afterwards, 1U/mg of RNase A was added. Each experiment was carried out with the same nasopharyngeal sample and 3-5 replicas. In each experiment a control with RNase incubation was added. The reaction was stopped by adding 400 μL of lysis solution and the RNA was extracted with the Quick-DNA/RNA Viral MagBead Kit (Zymo Research, USA). Five μL of the extracted solution was used for RT-PCR to measure the presence of SARS-CoV-2. The real-time RT-PCR protocol was adapted from Corman et al. 2020 (1). Briefly, a 20 μL reaction was prepared containing 5 μL RNA, 400nM primers, 200nM probe and 10 μL of 2 × reaction buffer provided with the iTaq universal Probe One-Step Kit (BioRad, USA). To quantify viral load, Cqs from the BioRad CFX96 system targeting the E gene were converted to viral load using a plasmid

containing the sequence of SARS-CoV-2 genes E and RdRp kindly provided by Jaime Castellanos' Virology Laboratory, Universidad del Rosario, Colombia.

Results:

To evaluate the virucidal properties of XYNTRUS, we designed a method to detect and quantify SARS-CoV-2 RNA directly from nasopharyngeal samples obtained from COVID-19 epidemiological surveillance in Barranquilla. RT-PCR positive samples for SARS-CoV-2 RNA were incubated in the presence of RNase to test whether enveloped viral particles, presumably infective, would protect the RNA from the enzymatic degradation. **Figure 1** shows that the viral RNA is stable in the untreated sample after the RNase treatment suggesting the envelope remains intact protecting the viral genome. We then treated viral samples with iodopovidone, a compound previously reported to have virucidal activity (2). **Figure 2** shows that a 1-minute treatment with a 4% solution of iodopovidone reduced the detection of viral RNA completely indicating that the envelope was compromised rendering the RNA susceptible to the RNase degradation.

To determine the action of Xyntrus®, we treated a SARS-CoV-2 positive sample with the solution as indicated above and quantified the RNA by qPCR after an RNase incubation. **Figure 3** shows a reduction of more than 5 logs in the viral load in the XYNTRUS-treated sample only in the RNase one. Next, we used the active ingredients found in several mouthwashes, D-Limonene (3%), Cetylpyridinium chloride (0,1%), and Chlorhexidine gluconate (10%), to determine virucidal activity over SARS-CoV-2. The three compounds showed reduction in the viral load, however, RNA was still detected as shown in **figure 4**.

Other commercial mouthwash solutions products where tested to determine the virucidal action. After the incubation with the different compounds followed by the RNase treatment, RNA was detected with similar titers suggesting that the nucleic acid was still protectd by the envelope (**Figure 5**).

In conclusion, under the conditions of our biochemical degradation assay where we tested the stability of the viral envelope to protect the RNA from RNase degradation

after treatment with different compounds, our results show that Iodopovidone and Xyntrus reduced the viral titer more than 6 logs, a reduction efficacy of more than 99,99%.

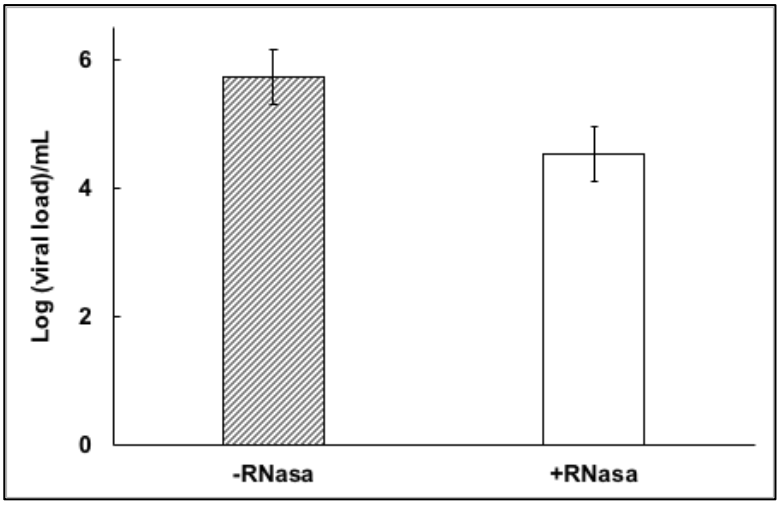


Figure 1. **RNase Treatment of Nasopharyngeal Sample Containing SARS-CoV-2.**

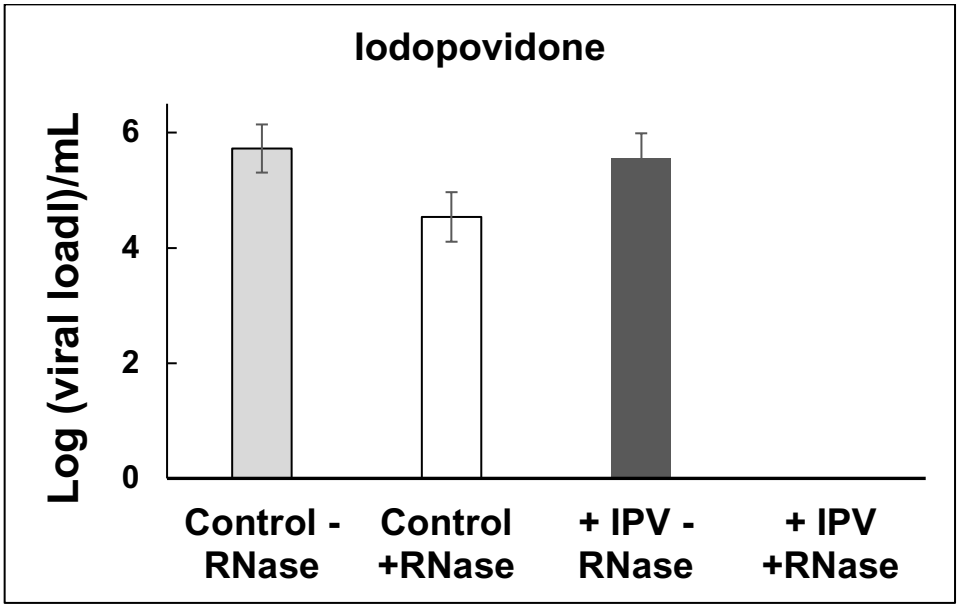


Figure 2. **Effect of Iodopovidone solution on the degradation of SARS-CoV-2 RNA**. Control= SARS-CoV-2 sample without treatment; Control +Rnase= SARS-CoV-2 sample without treatment with an RNase treatment.

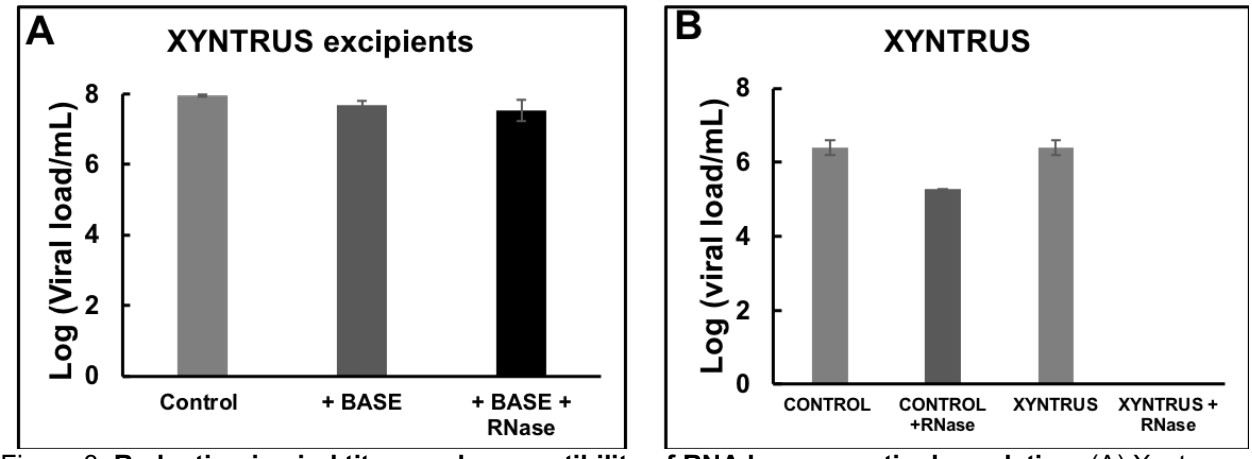


Figure 3. **Reduction in viral titers and susceptibility of RNA by enzymatic degradation.** (A) Xyntrus inactive compounds and excipients. (B) Xyntrus. Control= SARS-CoV-2 sample without treatment; Control +Rnase= SARS-CoV-2 sample without treatment with an RNase treatment.

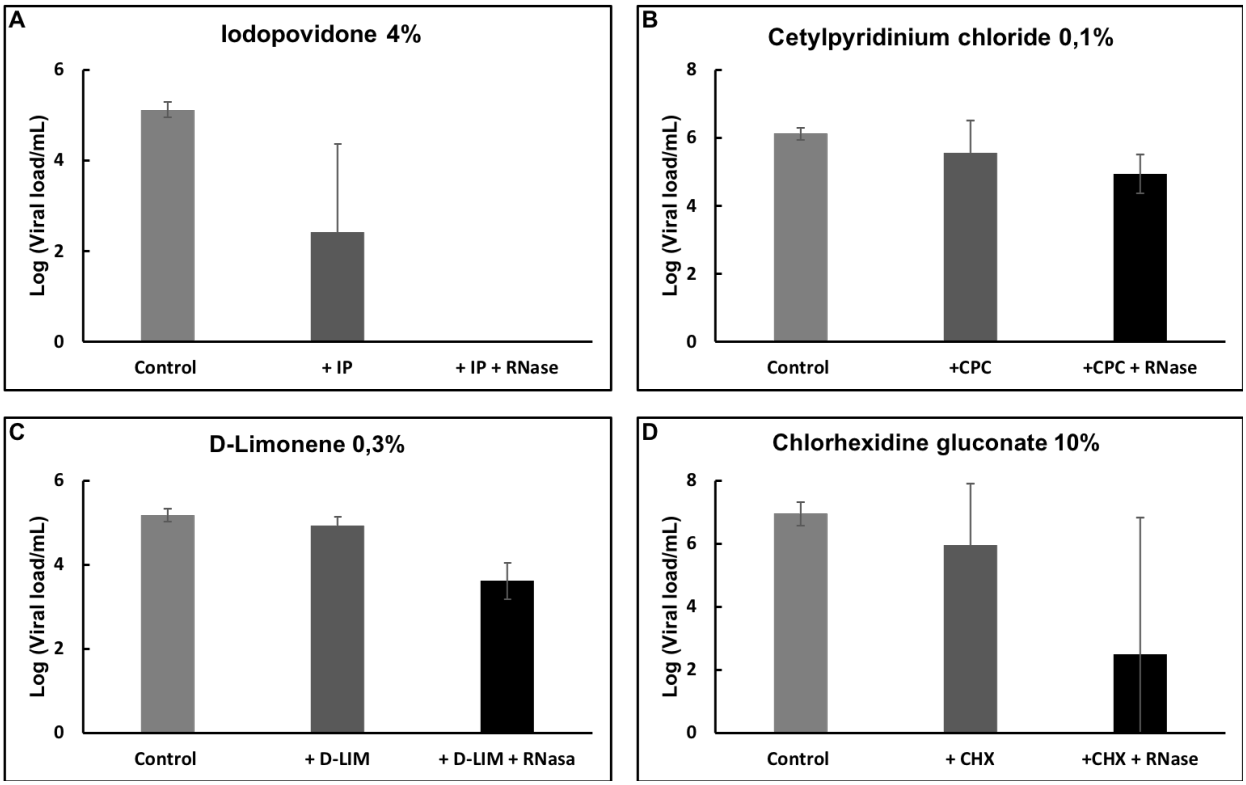


Figure 4. **Reduction in viral RNA titers by (A) Iodopovidone, (B) Cetylpyridinium chloride, (C) D-Limonene and (D) Chlorhexidine gluconate detected by RT-PCR.**

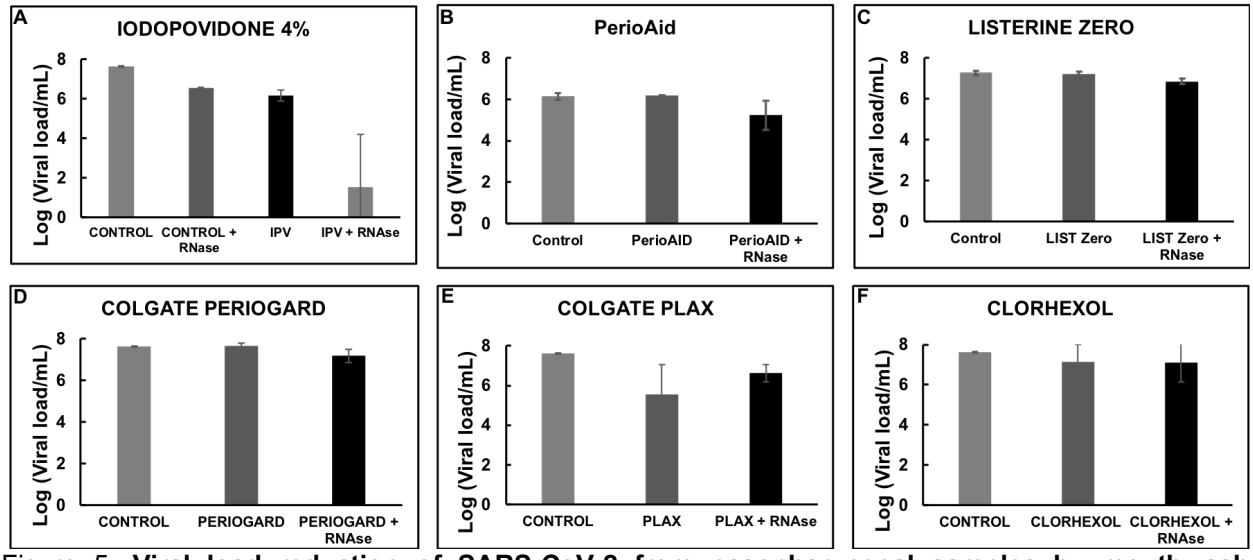
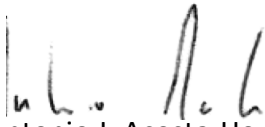


Figure 5, **Viral load reduction of SARS-CoV-2 from nasopharyngeal samples by mouthwash products.** (A) Iodopovidone; (B) Perioid; (C) Listerine Zero; (D) Colgate Periogard; (E) Colgate Plax; (F) Clorhexol. Control= SARS-CoV-2 sample without treatment; Control +Rnase= SARS-CoV-2 sample without treatment with an RNase treatment.

Experiments were carried by Yesit Bello, Msc.

Approved by:



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