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

Methodology: 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. 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 remains after the RNase treatment suggesting the envelope remains intact. We then incubated viral samples with different products to determine the virucidal action. After the incubation with the different compounds a RNase treatment was included to degrade RNA that was exposed if the envelope was degraded or compromised. We initially tested 4 products: XYNTRUS base (water, glycerin, citric acid, colorant, sodium citrate); 0.3% D-Limonene with EBE Technology™, an encapsulated

buffer containing essential oils; XYNTRUS (water, glycerin, sodium lauril sulfate, citric acid, mentha arvensis leaf oil, D-limonene, menthol, eucalyptus globulus leaf oil, methyl salycilate, cethylpiridinium chloride, colorant, sodium citrate); or 8 mg of iodopovidone. **Figure 2** shows the results with the 4 products where we observe a reduction of more than 99,9% with XYNTRUS similar to what is observed for iodopovidone, shown to have virucidal activity *in vitro* against SARS-CoV-2 (2). D-Limonene reduced the viral load up to 95%, being one of the main components of XYNTRUS mouthwash; however, it is the synergy of all of XYNTRUS elements that makes XYNTRUS efficacy greater than 99.9%.

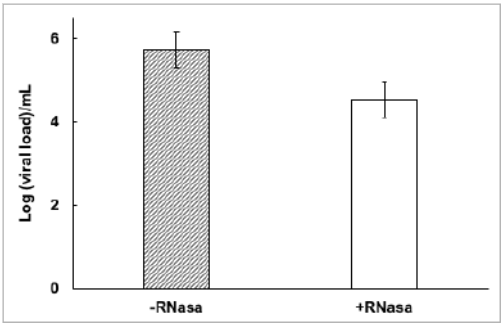

Figure 1. **RNase Treatment of Nasopharyngeal Sample Containing SARS-CoV-2.**

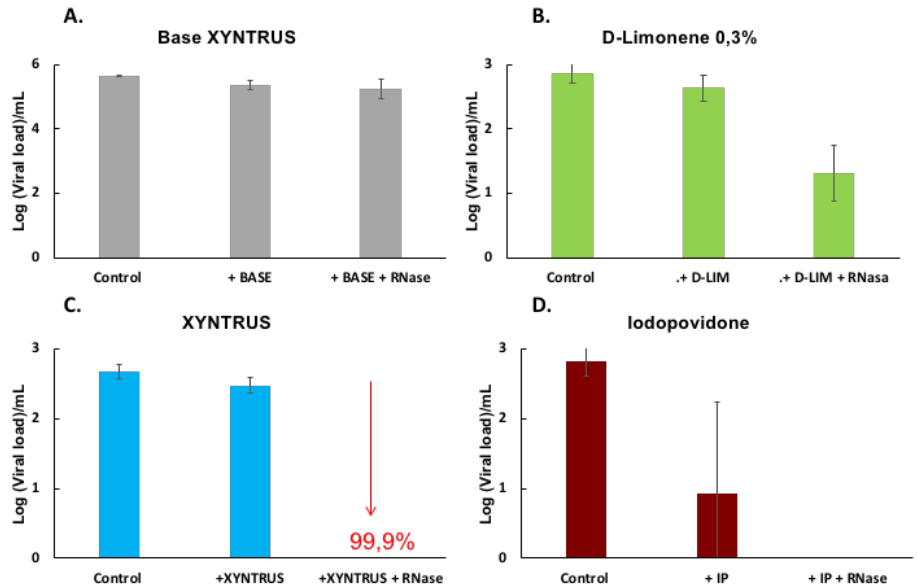

Figure 2. **Virucidal efficacy of oral rinses against SARS-CoV-2.** A. Xyntrus base. B. D-Limonene. C. XYNTRUS. D. Iodopovidone (IP).

Experiments were carried by Yesit Bello, Msc.

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