Poster # 2064

Effect of CPC Mouthrinses on Spike Protein Binding to ACE2 Receptor

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Objective

Evaluate the efficacy of cetylpyridinium chloride (CPC)-containing mouthrinses to:

- prevent the binding of the Receptor Binding Domain (RBD) of SARS-CoV-2 Spike protein with the human angiotensin-converting enzyme (ACE2) cell surface receptor protein *in vitro*.
- neutralize the SARS-CoV-2 Spike pseudotyped lentivirus in a cellular model.

Conclusions

All Colgate CPC-containing mouthrinse products tested are effective in reducing the binding of the Spike protein to ACE2 receptors *in vitro* and therefore, inhibit the viral entry into the ACE2-HEK cells.

Results

Figure 1. Inhibition of Spike: ACE-2 binding with Colgate Mouthrinse.

Inhibition of the binding of Receptor Binding Domain (RBD) of Spike protein with the ACE2 receptor with mouthrinse treatment. Percent inhibition of binding is calculated as reduction in chemiluminescence relative to the positive control (no product treatment).

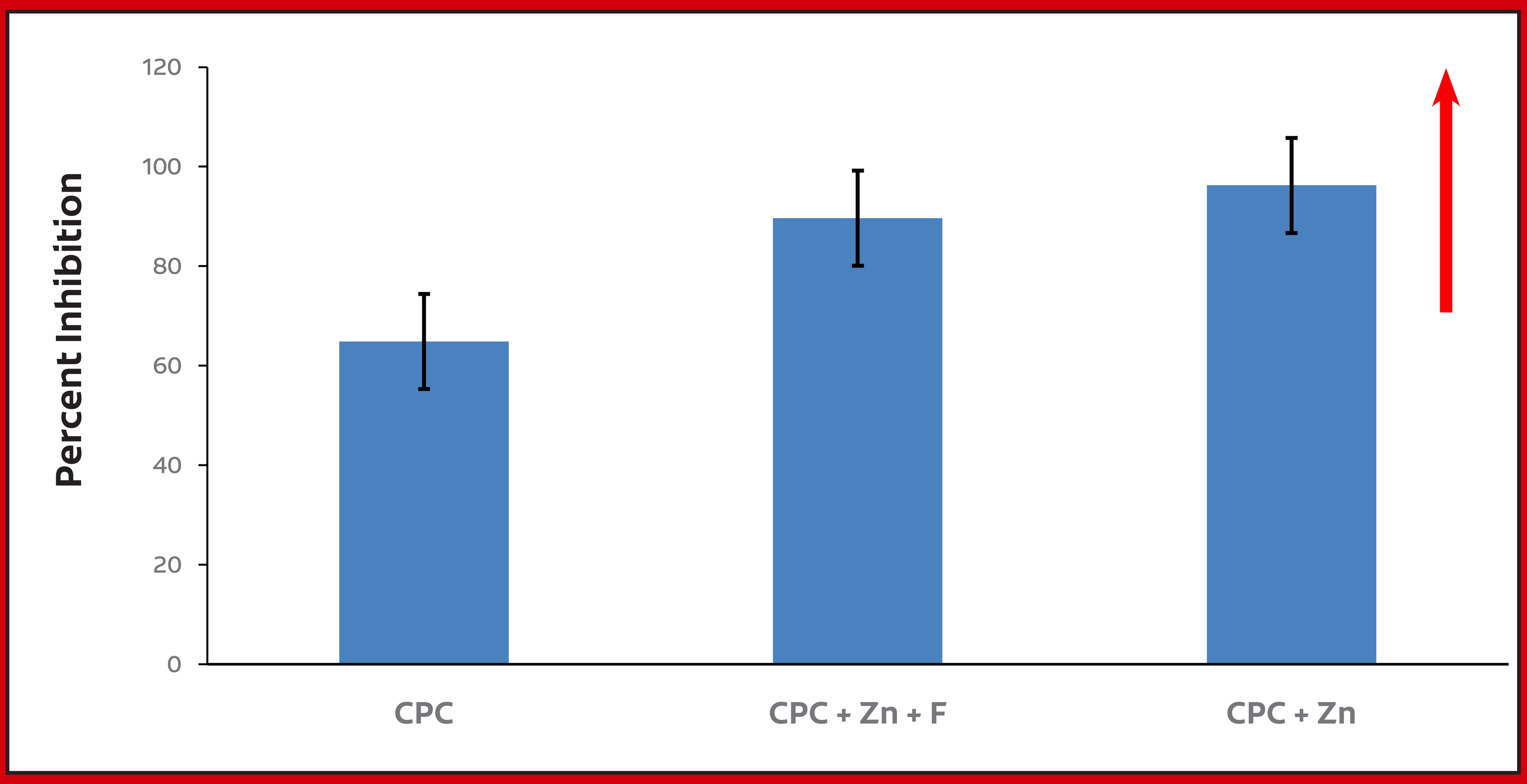


Figure 2. Neutralization of SARS-CoV-2 Pseudotyped Lentivirus with Colgate Mouthrinses.

Inhibition of SARS-CoV-2 Lentivirus entry into ACE2-HEK293 Cell lines after neutralization with mouthwash treatment. Positive control (CT) represents ACE2-HEK293 cells transduced with Lentivirus without any mouthrinse treatment, negative control (CT) represents cells not transduced with the lentivirus. HEK293 cell lines are shown for comparison where lack of ACE2 receptors result in poor transduction.

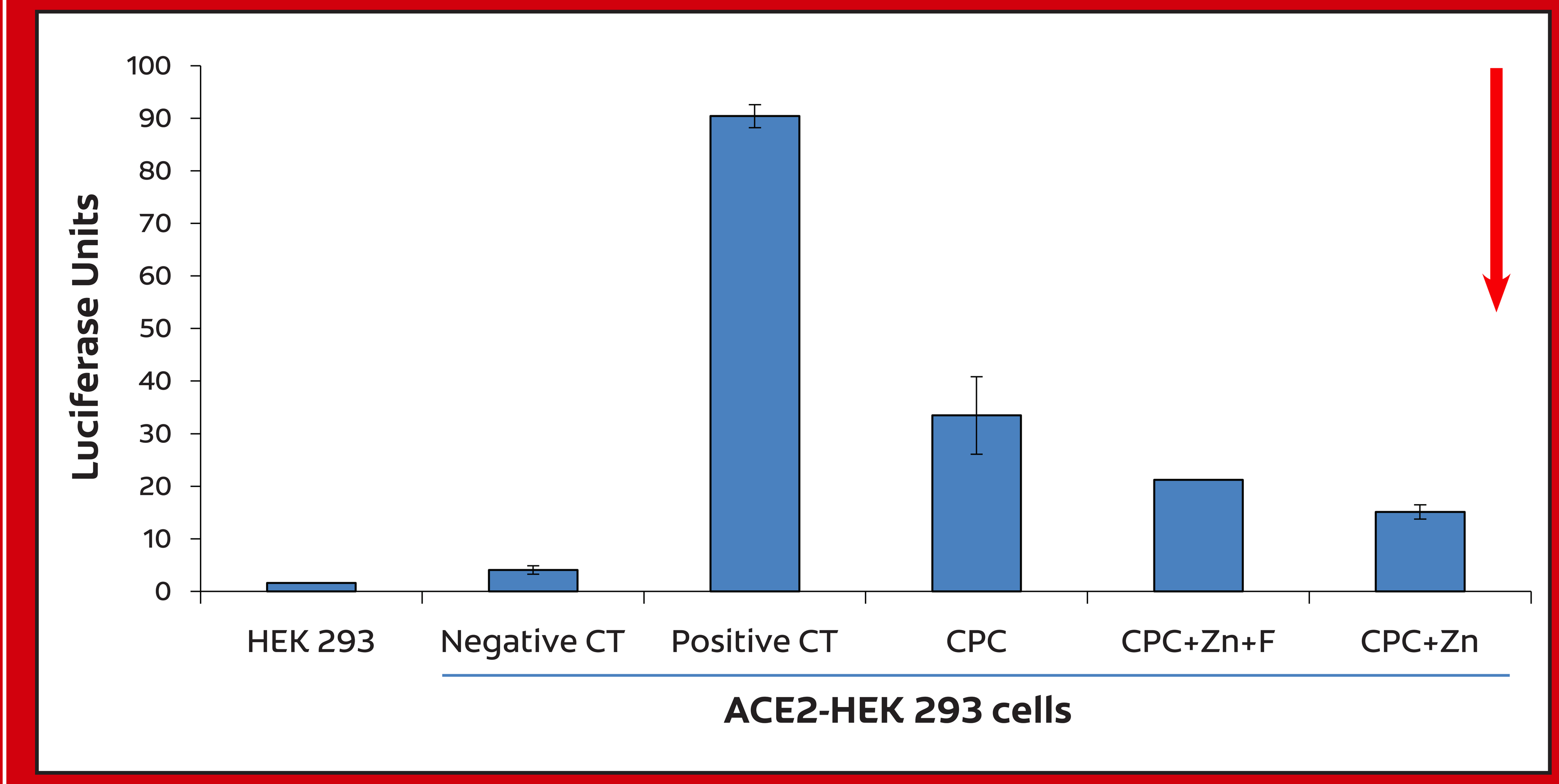
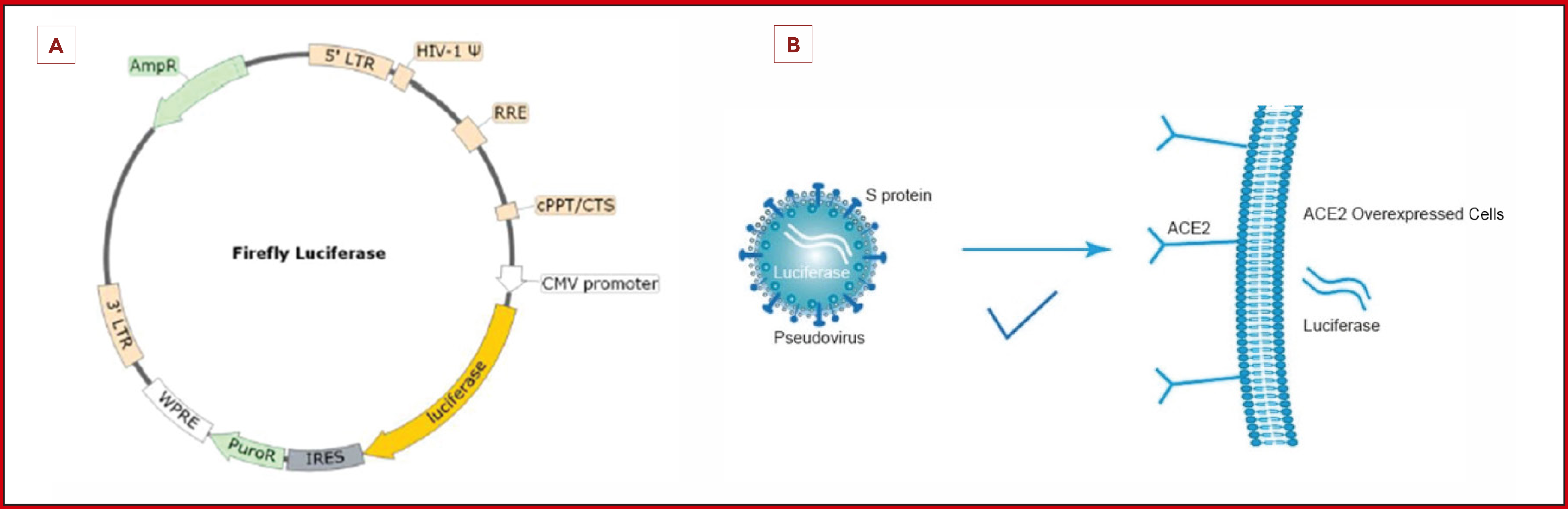


Figure 3.

A) Schematic diagram of the Luciferase (Luc) Reporter in SARS-CoV-2 Spike Pseudovirion.

B) Diagramatic representation of pseudovirus entry into ACE2-HEK cells and Luciferase expression.



Methods and Materials

- Spike-ACE2 binding inhibition assay:
  - Three Colgate mouthrinse products were tested using an ELISA-based approach.
  - 50 ng/well of SARS-CoV-2 Spike protein (RBD) solution was added into a 96-well plate and incubated overnight at 4C. After rinsing, each mouthrinse was added individually (3 replicates) and incubated for an hour.
  - 50 ng of ACE2-His protein was added to the plate and incubated for an hour.
  - The affinity of ACE2:Spike binding was quantified immediately by chemiluminescence.
  - The reduction in luminescence is proportional to the efficiency of the mouthrinses to inhibit the ACE2:Spike binding.

- SARS-CoV-2 lentivirus neutralization assay:
  - Approximately 10,000 cells/well of ACE2-HEK293 Recombinant or HEK293 cells (negative control) were seeded into a 96-well plate for overnight.
  - 50 μl of mouthrinse was preincubated with 5 μl of SARS-CoV-2-Spike pseudotyped Lentivirus (Luc reporter) for 30 minutes. After incubation, 55 μl of virus/mouthrinse mix was added into the remaining 45 μl of media in each well.
  - After 18 hrs of transduction the medium was replaced with a fresh medium.
  - Luciferase activity was quantified after 48 hrs of transduction.
  - Reduction in luciferase activity is a measure of mouthrinse efficacy in preventing entry of lentivirus into the cells.
  - Detailed protocols for both methods are provided in the Supporting Material[1,2].

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