

Proof of Concept (POC) experiment – Enviro-Biotics® by betterair against Pseudo Covid 19

**Pseudo Virus – Model for lab use to prove control over Covid 19*

The following is a step by step account of the experiment conducted at University of Genoa, Italy in order to prove the important first stage probability of Covid 19 reduction on surfaces by using Natural Environmental Probiotics (Enviro-Biotics®).

Actions:

- Activation of the spores into active bacteria was performed according to the agreed protocol (see Fig .1)
- After 4h of bacteria growth in 100 ml of specific medium, the suspension was cooled under agitation for 10 minutes. The bacteria growth was done in duplicate. Final OD at 600 nm of both suspensions was 0.77.
- The suspensions were centrifuged at 4000xg, pellets of active bacteria were collected and unified, washed once with saline buffer and re-suspended in 20 ml of saline buffer (concentrated x10 fold as requested). The final bacterial suspension was added to a sterile sprayer.
- The active bacteria were sprayed three times on a sterile plastic surface under a switched-off hood (temperature 26 °C, .RH 78%) and then the pseudocovid-19 was added. This step was performed for each time point of the experiment (0, 1, 3.5 and 7 h). In parallel, as control, the same amount of pseudocovid-19 was seeded on another plastic surface (for each time point) in the absence of bacteria. A negative control

with only sprayed bacteria on a plastic surface was also performed.

- At the end of each time point; 1) the pseudocovid-19 alone, 2) pseudocovid-19 mixed with the active bacteria and 3) bacteria alone, were recovered from the plastic surfaces, centrifuged and filtrated to remove all bacteria. For each time point, the supernatants obtained after the centrifugation and filtration of all samples and controls were put on Hep G2 cells, seeded in 96- well plates, for approx. 72 hours.
- After the above incubation time (72 hours), confocal microscopy acquisition of Hep G2 cells infected with the pseudocovid-19 recovered from each time point of the experiment were performed (Fig. 2).
- The same cells (for each different time point) used for the confocal microscopy acquisition were then measured for their fluorescence by spectrofluorometer for quantifying the residual infectivity of the pseudocovid-19 (directly proportional to the measured fluorescence) after its incubation with or without the active bacteria in the time course (Fig. 3)

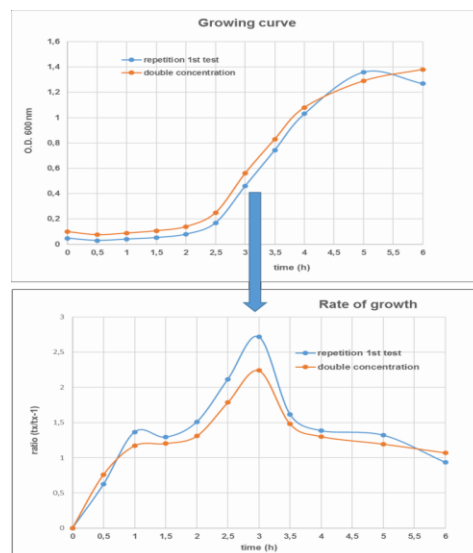


Fig. 1 Graphs of the tests performed to set the optimal duration for spore activation..

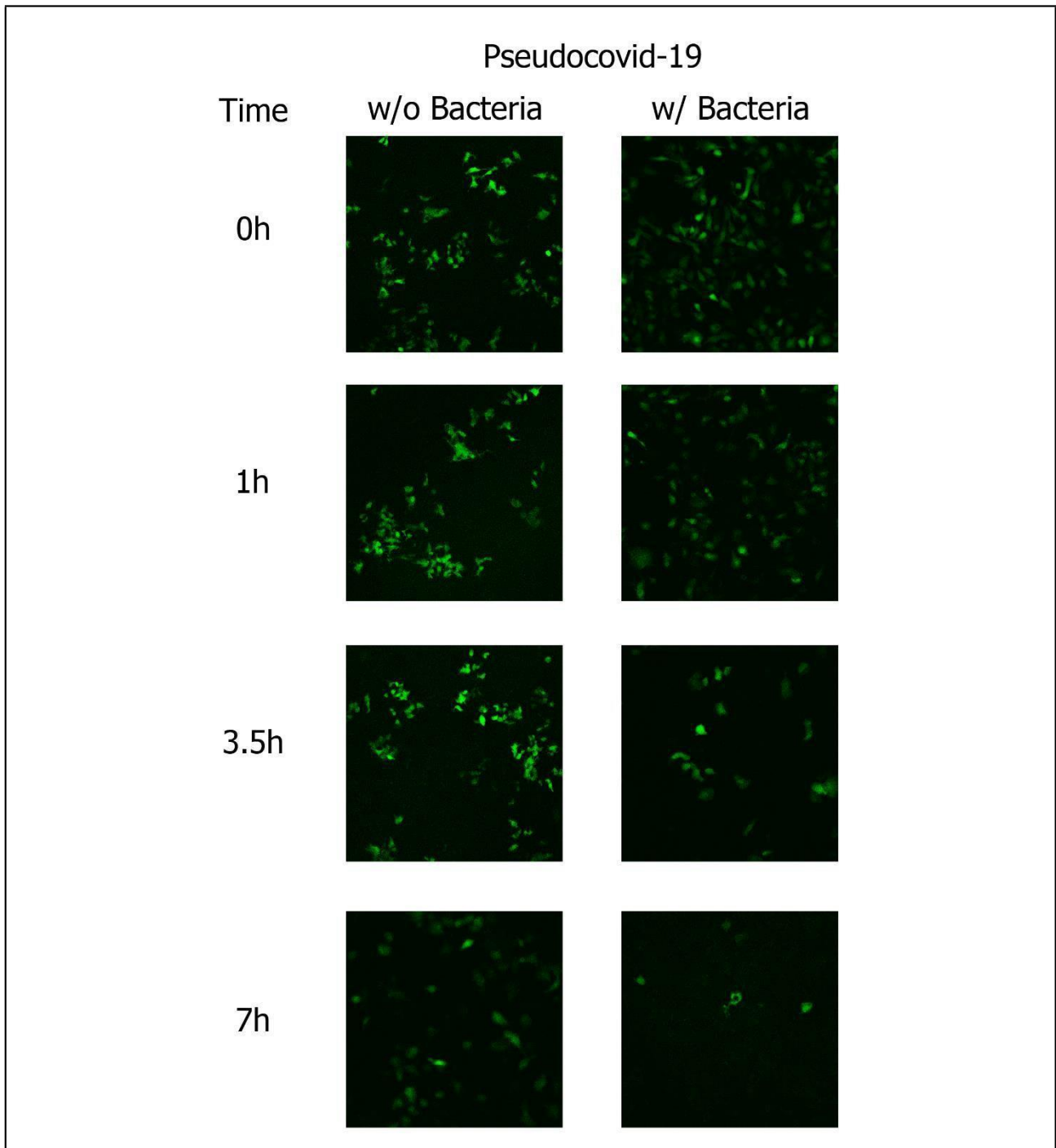


Fig. 2 Confocal microscopy analysis of Hep G2 cells infected with pseudocovid-19 previously incubated with or without active bacteria at different time points.

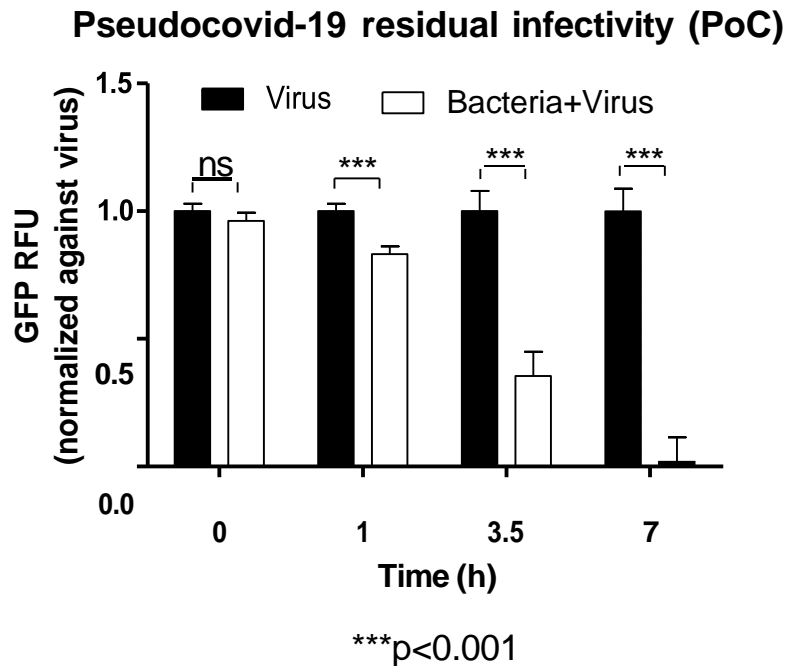


Fig. 3 Fluorimetric quantitation of pseudocovid-19 residual infectivity detected as GFP fluorescence. Fluorescence values were normalized to untreated pseudocovid-19 at each time point. Background deriving from auto-fluorescence of cells alone was subtracted at each time point. P value was calculated by unpaired t-Test.

Conclusion:

It is safe? to conclude (It can be concluded?) at this point that Enviro-Biotics® clearly reduce the Pseudocovid 19 on the tested surfaces. (Fig 2)

Additional experiments are underway.