



Epithelix

in vitro Solutions for Respiratory Diseases and Chemical Testing

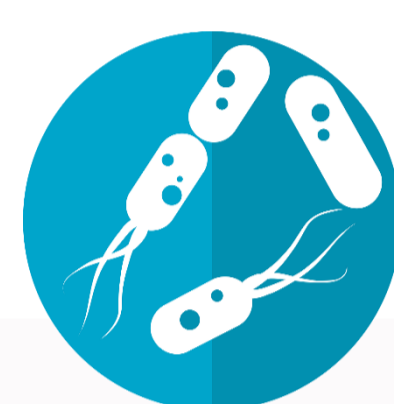


In vitro evaluation of novel antibiotics against *Pseudomonas aeruginosa* infection on Human Airway Epithelia

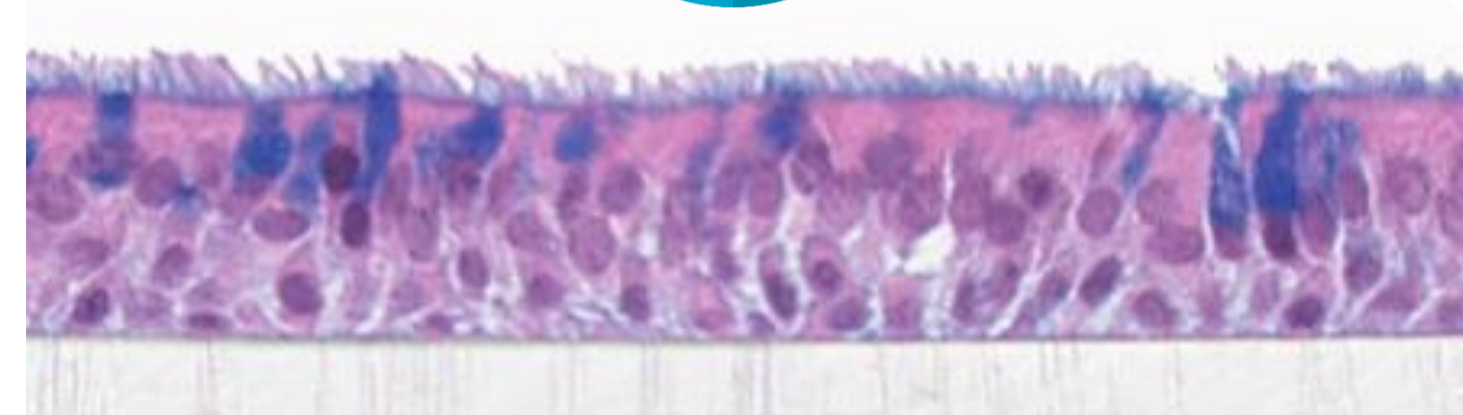
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Pseudomonas aeruginosa (PA) is a common Gram-negative bacterium. PA's infections are increasingly associated with acute exacerbations in chronic obstructive pulmonary disease (COPD). We report herein the use of 3D airway epithelia, MucilAir™ - pool of donors, made of a mixture of nasal primary cells from 14 human donors for screening novel antibiotics upon PA infection. PA was inoculated ($1E+02$ CFU/ 0.33 cm^2) on fully differentiated MucilAir™ in presence or absence of mucus, with or without Meronem ($50\text{ }\mu\text{g/ml}$). PA growth, cilia beating frequency (CBF), cytotoxicity (LDH) and tissue integrity (TEER) were assessed daily during 4 days.

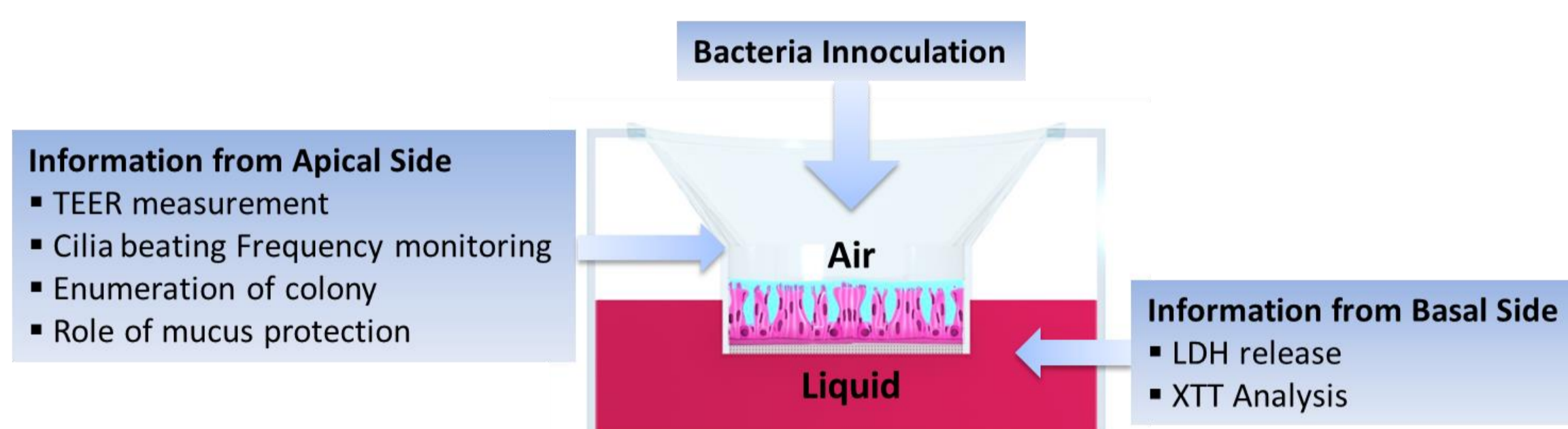
A higher proliferation rate of PA in absence of mucus was observed, highlighting the protective role of mucus containing antimicrobial peptides. Meronem efficiently inhibited both growth of PA and the cytotoxicity (LDH) and restored the impaired barrier function (TEER) in a time dependent manner.



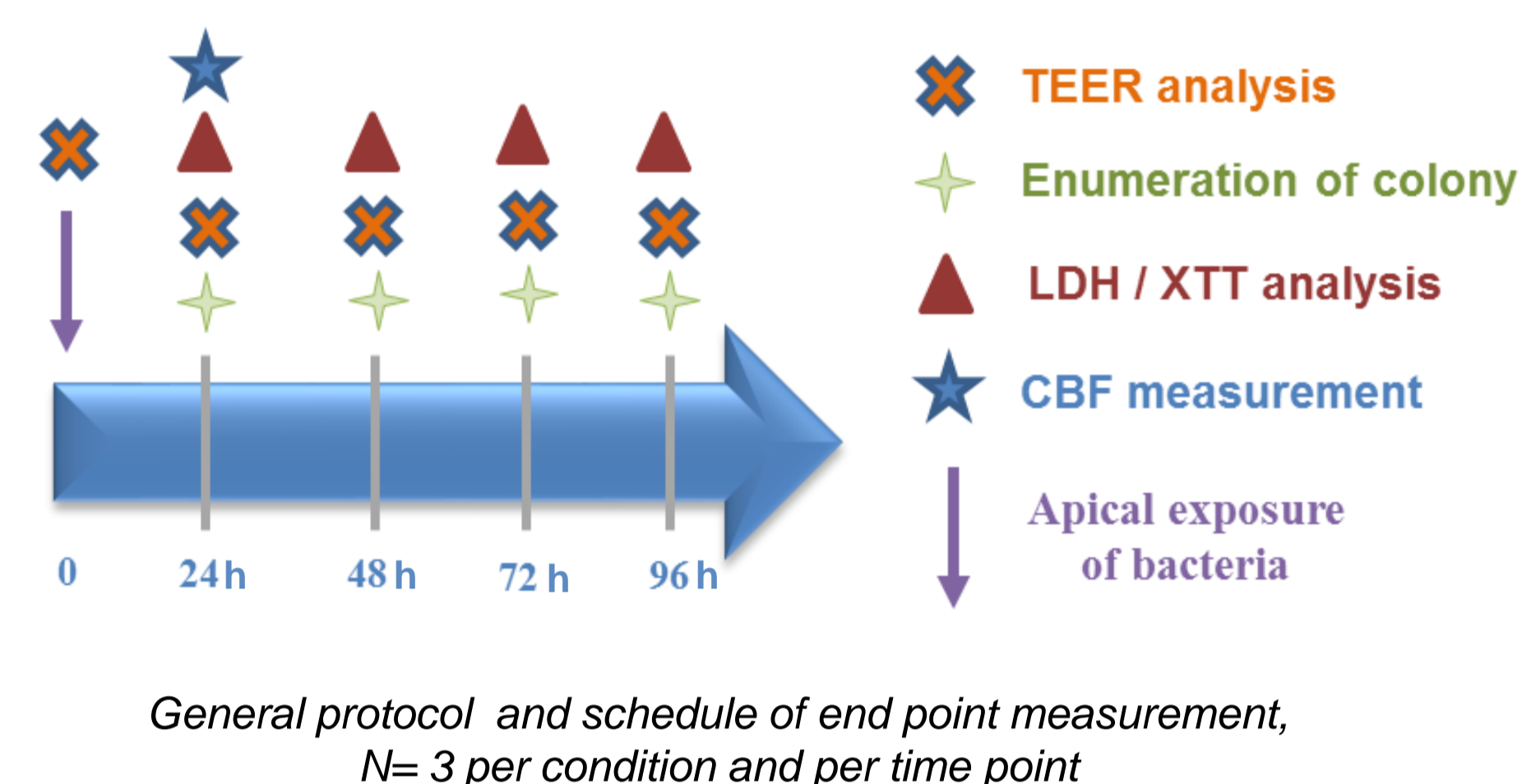
Testing Strategy



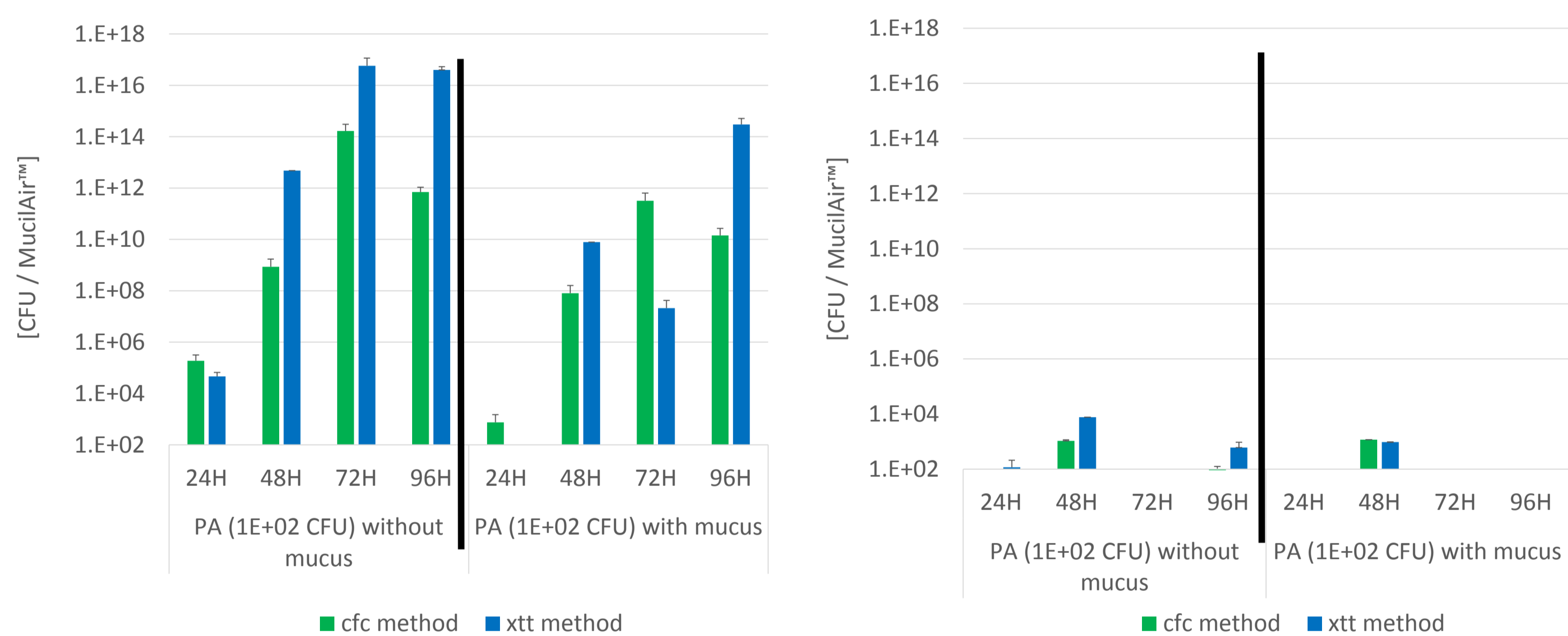
MucilAir™, a fully differentiated 3D *in vitro* cell model of the human airway epithelia. The cells were freshly isolated from the biopsies. After 45 days of culture at air-liquid interface, the epithelia were fully differentiated, both morphologically and functionally.



Pseudomonas aeruginosa (PA) was inoculated on MucilAir™-pool of donors ($1E+02$ CFU/ 0.33 cm^2) on apical side. Toxicity induced by infection is monitored by several non-destructive endpoints such as TEER measurement, cilia-beating frequency monitoring and LDH releases in presence or absence of mucus

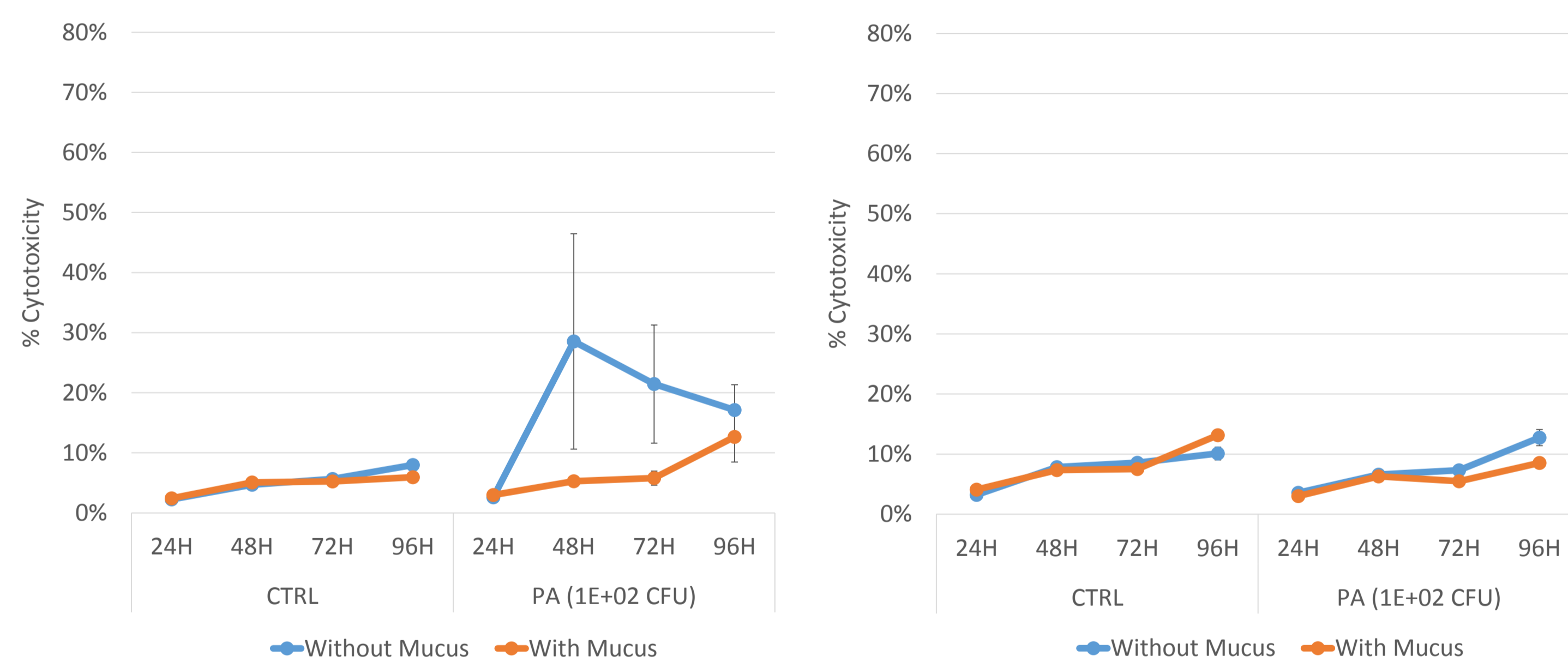


Quantification of PA'growth on MucilAir™ without (left) and with (right) Meronem



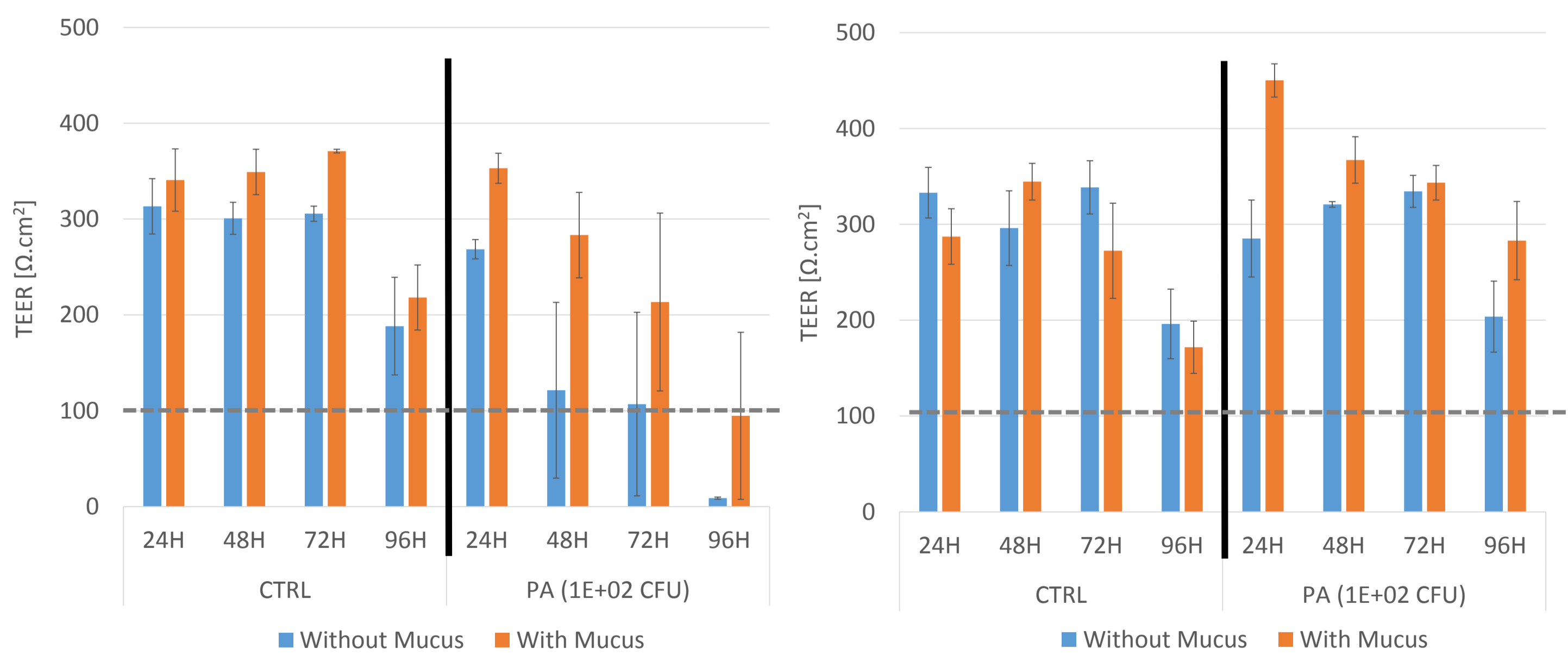
- Mucus slightly inhibited PA growth.
- Meronem efficiently inhibited PA growth.

Cytotoxic effect of PA infection on MucilAir™ without (left) and with (right) Meronem



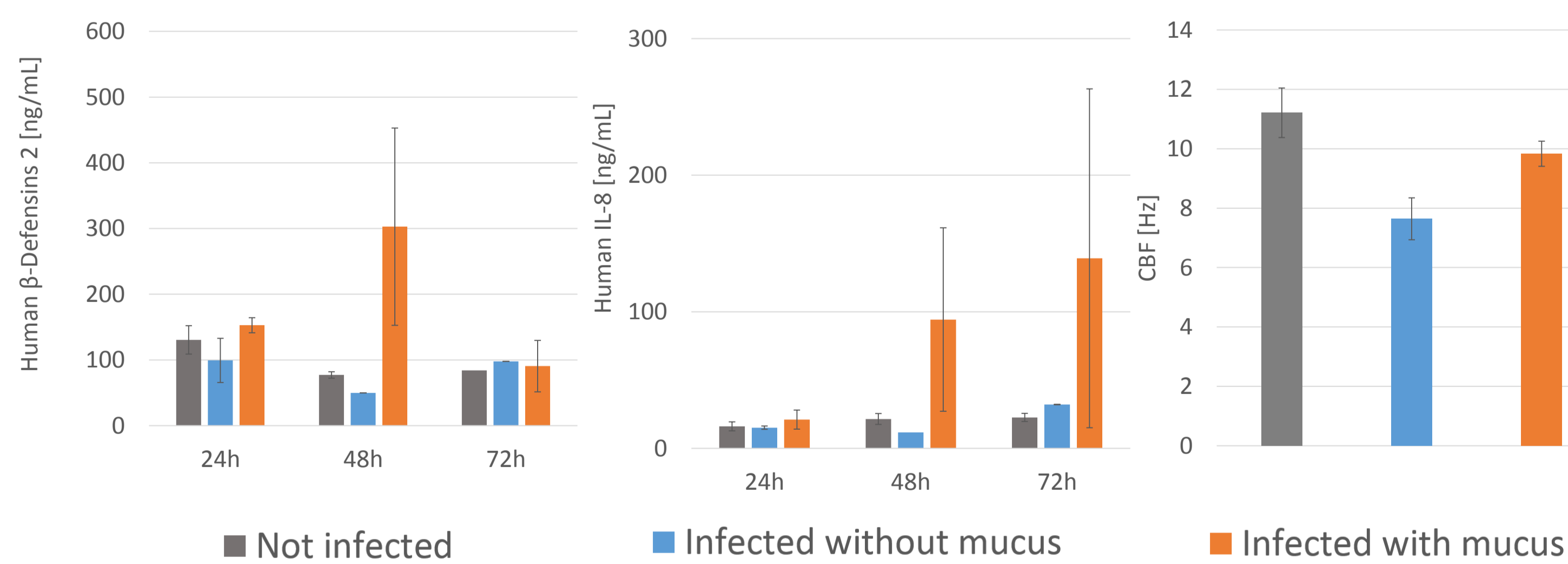
- Mucus seemed to prevent cytotoxicity induced by PA up to 72h
- Meronem prevented cytotoxicity induced by PA up to 72h.

Evaluation of barrier function on MucilAir™ after PA infection without (left) and with (right) Meronem



- PA impaired the barrier function from 48h post inoculation without mucus.
- The presence of mucus protected the barrier function up to 48h.
- Meronem efficiently preserved the barrier function from PA infection

Cilia Beating Frequency, IL-8 and β-Defensin 2 are not affected by PA infection



- PA didn't affect cilia beating frequency at 24h.
- No significant difference was found in IL-8 and β-Defensin 2 secretion between the tissues infected or not by PA.

Conclusion

- 1) Mucus inhibits PA growth and protects the barrier function up to 48h.
- 2) Meronem efficiently inhibits PA growth, preventing cytotoxicity and loss of tissues integrity.
- 3) These results demonstrate that MucilAir™ is a robust, reliable and relevant tool for drug development against PA infection.

