# Development of new mean of preservation of human corneas for transplantation with antifungal action spectrum

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## Introduction

Corneal transplantation is, in general, a very successful procedure. However, there is a growing incidence of very severe post-transplant infectious complications of fungal origin (keratitis and endophthalmitis), whose diagnosis and treatment can be complex. n this way, they are compromising the success of the procedure and the patients' vision. In view of the importance of this topic, as part of the general objective of the doctoral project, we determined the action potential of the triple combination of two antifungal agents already used together with a potential antifungal agent to be used against *Fusarium* strains, a genus commonly related to these infections.

## Experimental section

The three-dimensional Checkerboard assay combining voriconazole, natamycin and clioquinol was performed according to Stein *et al*. (2015), with modifications. The wells of a microplate were filled with 100 µl of solution containing the three antifungal agents in different combinations of concentrations and 100 µl of the fungal inoculum. For each fungal strain and antifungal agent, the concentration ranges tested were selected from previously determined minimum inhibitory concentrations (MICs). The concentrations tested were: MICx2; MIC; MIC/2, MIC/4 and MIC/8 for each antifungal agent. The microplates were incubated at 32° C for 48 hours. The fractional inhibitory concentration index (FICI) for triple combination of antifungal agents was calculated following the formula:

$$FICI = \frac{MIC_A \text{ in combination}}{MIC_A \text{ alone}} + \frac{MIC_B \text{ in combination}}{MIC_B \text{ alone}} + \frac{MIC_C \text{ in combination}}{MIC_C \text{ alone}}$$

## Results and Discussion

Voriconazole and natamycin are antifungal agents used in the treatment of eye infections, however, the therapeutic response is not always positive, even when combined. The triple combination (voriconazole, natamycin and clioquinol) showed action potential against *Fusarium* strains, since the synergistic interaction was observed in 60% of them (Table 1).

## Conclusions

The combination of the usual antifungal agents (voriconazole and natamycin) with this potential antifungal agent (clioquinol) for the treatment of eye infections caused by fungi of the Fusarium genus represents a promising therapeutic option. The number of strains tested must be expanded and toxicity studies carried out to ensure the efficacy and safety of this combination.

## Acknowledgments

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## References

1. C. Stein, O. Makarewicz, J.A. Bohnert, Y. Pfeifer, M. Kesselmeier, S. Hagel & M.W. Pletz. Three Dimensional Checkerboard Synergy Analysis of Colistin, Meropenem, Tigecycline against Multidrug-Resistant Clinical *Klebsiella pneumonia* Isolates. PLOS ONE, **10**, 6 (2015).

TABLE 1. Minimal Inhibitory Concentration (MIC) for voriconazole, natamycin and clioquinol tested alone and in combination against *Fusarium* strains.

| **STRAINS** | **MIC (µg/mL)** | | | **MIC COMBINATION (µg/mL)** | | | | |
|---|---|---|---|---|---|---|---|---|
| | **VOR** | **NAT** | **CLIO** | **VOR** | **NAT** | **CLIO** | **FICI** | **INTERACTION** |
| ATCC 36031 | 128 | 16 | 1 | 16 | 2 | 0.5 | 0.750 | SYN |
| HCF46 | 4 | 16 | 1 | 1 | 4 | 1 | 1.500 | IND |
| F9 | 128 | 16 | 1 | 64 | 2 | 0.25 | 0.875 | IND |
| F21 | 128 | 16 | 1 | 8 | 2 | 0.5 | 0.687 | SYN |
| F28 | 64 | 16 | 1 | 8 | 2 | 0.25 | 0.500 | SYN |

*Fusarium solani*: ATCC 36031, F28; *Fusarium oxysporum*: HCF46; *Fusarium falciforme*: F9; *Fusarium keratoplasticum*: F21.
MIC: Minimal Inhibitory Concentration.
VOR: voriconazole; NAT: natamycin; CLIO: clioquinol.
SYN (Synergism): FICI ≤ 0.75; IND (Indifference): 0.75 < FICI ≤ 4; ANT (Antagonism): FICI> 4.