Course

- **Demonstrative or interactive (will be depend on the numbers of people)**
- **2 days – 3 hours each day**
- **September 06 (theoretical)**
- **September 07 (practical)**

Material

-Micropipettes
20, 100 and 200 µL
-Multichannel
100 µL
-Media Culture
100 mL of the BHI broth (autoclaved) or equivalent broth for *Staphylococcus aureus* or *Escherichia coli*
-Bacteria
Staphylococcus aureus or *Escherichia coli*
-Incubator
37ºC

Method (Protocol)

Chequerboarder synergy assay

The interaction between compounds and the treatment drugs was conducted against MTB $H_{37}Rv$ (ATCC 27294), according by Luna Herrera et al. (2007) [1] with some modifications described by Moody (1992) [2]. For the assay, combinations of the two compounds (2D) (ex.: Compound (1) and amoxicilin) was used in 96-well microtiter plate (NUNC^{tm}), where the compound test (A) was transferred to row A (X axis) of the columns 2-9 in concentration 4 times higher than MIC alone and after two-fold dilutions were made to row H. The compound (B) (first-line drugs) was transferred to the column 2 (Y axis) of rows A-H in concentration 4 times higher than MIC alone and after two-fold dilutions were made to column 8. The column 10 was used as positive control (growth bacteria) and column 11 as negative control (only media). Every time that the assay was made, together, was evaluated the alone MIC for the compounds A and B. After the crossing between the drugs, MTB $H_{37}Rv$ (ATCC 27294) was thawed and added, yielding a final testing volume of 200 µL with $2x10^4$ CFU/mL. Microplates were incubated for 7 days at 37°C, after a mixture of Alamar Blue solution (20 µL) (Trek Diagnostics,

Westlake OH, USA) and sterile 10% Tween 80 (12 µL) was added. The fluorescence was read (530 nm excitation filter and 590 nm emission filter) in a SPECTRAfluor Plus (Tecan®) microfluorimeter [3]. The results were analyzed using the Fraction Inhibitory Concentration (FIC) (**scheme 1**), where the lowest FIC value between all wells with 90% inhibition of growth of the MTB was evaluated [2]. The FIC follow these interpretations: ≤ 1 synergism; >1-4 indifferent; >4 antagonisms [4]. Each test was set up in triplicate and no statistical significance was observed.

$$\textbf{FIC} = \frac{\text{MIC } \textbf{[A]} \text{ combinated}}{\text{MIC } \textbf{[A]} \text{ alone}} + \frac{\text{MIC } \textbf{[B]} \text{ combinated}}{\text{MIC } \textbf{[B]} \text{ alone}} = \textbf{FIC index}$$

Scheme 1: Calculation of Fraction Inhibitory Concentration (FIC)

[1] LUNA-HERRERA, J.L.; COSTA, M.C.; GONZALEZ, H.G.; RODRIGUES, A.I.; CASTILHO, P.C. Synergistic antimycobacterial activies of sesquiterpene lactones from *Laurus spp*. **J Antimicrob Chemother** 59; 548-552, 2007.

[2] MOODY J.A. Synergism testing: broth microdilution checkerboard and broth macrodilution methods. In: Isenberg HD, editor. Clinical microbiology procedures handbook. Washington, DC: American Society for Microbiology, p.5.18.1–28, 1992.

[3] PAVAN, F.R.; POELHSITZ, G.V.; DO NASCIMENTO, F.B.; LEITE, S.R.A.; BATISTA, A.A.; DEFLON, V.M.; SATO, D.N.; FRANZBLAU, S.G.; LEITE, C.Q.F. Ruthenium (II) phosphine/picolinate complexes as antimycobacterial agents. Eur J Med Chem 45; 598-601, 2010.

[4] POETA, M.D.; CRUZ, M.C.; CARDENAS, M.E.; PERFECT, J.R.; HEITMAN, J. Synergistic antifungal activities of Bafilomycin A1, Fluconazole, and the Pneumocandin MK-0991/Caspofungin Acetate (L-743,873) with calcineurin inhibitors FK506 and L-685,818 against *Cryptococcus neoformans*. **Antimicrob Agents Chemother** 44; 739-746**,** 2000.