Method Optimization for High-throughput Antifungal Screening

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INTRODUCTION:

A standard antifungal susceptibility test in vitro has to be optimised for subsequent transfer to high-throughput robotic system assay. Numerous factors can influence the MIC (minimal inhibitory concentration) values in antifungal testing, such as inoculum size, pH, medium composition, temperature and duration of the incubation, as well as the standard and solubility of standard antifungal compounds. In this study, MIC values were determined for three different fungal species (Candida albicans, Candida krusei and Saccharomyces cerevisiae). Three antifungal substances (amphotericin B, fluconazole, mycomazole) were used as standards.

Optical density was measured at 600 nm by spectrophotometer TECAN, Ultra after 24 and 48 hours of incubation. The MIC end-point for the amphotericin B has been defined as the minimal concentration in which 99.9% of growth inhibition could be observed. Results for the other two antifungal drugs represented the lowest concentration where 95% of growth inhibition was observed, as compared to growth control. In parallel, the neutrophilic measurements of the same plates were made on Neubauer's Acetan-thrombocytes. The number of neutrophils was determined using a hemacytometer. The number of neutrophils in each well was counted at 400× magnification.

All tests were performed in 96-well plate format and were automated for the TECAN Genios 153 robot system.

MATERIAL AND METHODS:

Test organism:
C. albicans F0429
C. albicans F0430
C. albicans F024
C. albicans ATCC 90028
S. cerevisiae

Antimycotic:
Amphotericin B (USP)
Fluconazole (Pfizer)
Mycomazole (USP)

Media:
RPMI 1640 pH 7.0 (buffered with MOPS)
YNGE (pH 7.0)
YNGE (pH 6.0)

Antifungal susceptibility testing:

The MIC of each antifungal was determined against test isolates by using both microdilution technique as described by the National Committee for Clinical Laboratory Standards (NCCLS). The starting inoculum was approximately 1x10⁴ CFU/ml. Microtiter plates were incubated at 37°C and MICs were recorded after 24 and 48 hours of incubation. The susceptibilities of isolates for antifungals were defined as the lowest drug concentration which resulted in a 95% and 99.9% reduction of growth compared with the growth of untreated controls (MIC₅₀ and MIC₉₉.₉). The results were presented in tables for each experiment.

MIC values after 24 hours of incubation comparison of spectrophotometry and nephelometry read-out. After 24 hours incubation read-out values of MICs had no differences for all four tested compound.

MIC values after 48 hours of incubation comparison of spectrophotometry and nephelometry read-out. Differences in MIC values are blue marked. Afer 48 hours incubation differences were mainly in MICs values for fluconazole.

CONCLUSIONS:

1. Nephelometry could be the quick method for MIC determination in HTS, but the panel of test strains should be composed of susceptible and resistant strains for different control substances in the same run. This is important because the substances could be compared with control substances and according to this divided in active and non-active groups.

2. The phenotype of tested strain is very important and should be controlled before each run of testing.

3. It is very good method for quick determination of fungic-static and fungicidal substances. (See curves for amphotericin B).

4. According to NCCLS media for anti-fungi testing is RPMI 1640 pH 7.0, but sometimes activity of some compounds is better on YNG pH 6.0, so in some cases the parallel runs should be made.

5. According NCCLS, the incubation period is 48 hours, but we recommend also read-out after 24 hours especially in primary screening.

LITERATURE: