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Comparison of Caspofungin MICs by Means of EUCAST Method EDef 7.1 Using Two Different Concentrations of Glucose▽

Juan Luis Rodriguez-Tudela,[1]* Alicia Gomez-Lopez,[1] Maiken C. Arendrup,[2] Guillermo Garcia-Effron,[3,4] David S. Perlin,[4] Cornelia Lass-Flörl,[5] and Manuel Cuenca-Estrella[1]

Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain[1]; Unit of Mycology and Parasitology, Statens Serum Institut, Copenhagen, Denmark[2]; Laboratorio de Micología, Universidad Nacional del Litoral, Ciudad Universitaria-Paraje el Pozo, Santa Fe, Argentina[3]; Public Health Research Institute, University of Medicine and Dentistry-New Jersey Medical School, Newark, New Jersey[4]; and Department of Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria[5]

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According to the product insert for Cancidas (caspofungin acetate), the drug must not be diluted in solutions containing glucose as this decreases caspofungin stability. The aim of this study was to compare caspofungin MICs for a collection of yeasts by means of EUCAST method EDef7.1 but using two different concentrations of glucose: 2% versus 0.2%. MICs were identical or within one 2-fold dilution for 93 out of 95 strains (97.9%), showing that glucose does not interfere with susceptibility.

In a previous work, a comparison of the major susceptibility testing methods for echinocandins was performed using a well-characterized panel of *Candida* strains (1). EUCAST method EDef 7.1 performed as one of the best methods for discriminating wild-type strains from isolates with mutations in *FKS* hot spot regions. However, for caspofungin the MICs obtained by EUCAST method EDef 7.1 were in general higher than those obtained using the CLSI M27A3 methodology. In addition, the number of very major errors (number of *FSK* hot spot mutants classified as susceptible according the wild-type upper limit values) was 50% for EUCAST method EDef 7.1 (1), while 7% of very major errors were obtained by means of CLSI M27A3 at 24 h (1, 2). Finally, this previous work also found that caspofungin MICs obtained by means of EUCAST method EDef 7.1 and CLSI M27A3 were, in general, higher than those obtained by Pfaller et al. in a previous work using CLSI M27A3 methodology (4), indicating a possible variability associated with caspofungin antifungal susceptibility testing (1). Thus, it was of interest to explore which variables influence the reproducibility of the susceptibility tests for caspofungin. Although it has been proven that both the EUCAST EDef 7.1 and CLSI M27 A3 (2, 5) methodologies generate similar results for amphotericin B and azole drugs (3, 6), this does not appear to be the case for caspofungin. The main differences between EUCAST method EDef 7.1 and CLSI M27A3 include different inocula (10^5 versus 10^3 CFU/ml, respectively) and glucose concentrations (2% versus 0.2%, respectively) (3, 6).

The product insert for Cancidas states that the compound must not be diluted in solutions containing glucose because it decreases drug stability. As stated previously, the glucose concentration in the growth medium recommended for method EDef 7.1 in RPMI 1640 is 10 times higher than that recommended by the CLSI (5). As this concentration could have influenced the MIC values obtained by means of EUCAST method EDef 7.1 (5), the Antifungal Susceptibility Testing Subcommittee of EUCAST decided to set up a comparison test to examine whether the increased glucose concentration affects the MIC results. The test compared caspofungin MICs for a collection of well-characterized yeast isolates by means of EUCAST method EDef 7.1 (5) but using growth medium with two different concentrations of glucose: 2% versus 0.2%.

Strains. Ninety-five strains of *Candida* were used throughout the study. Sixty-seven were considered susceptible and 28 resistant, including 10 *FKS* wild-type and 10 *fks* hot spot mutant *Candida albicans* isolates, 9 *FKS* wild-type and 10 *fks* hot spot mutant *Candida glabrata* isolates, 1 *FKS* wild-type and 1 *fks* hot spot mutant *Candida dubliniensis* isolate, 13 FKS hot spot wild-type and 3 *fks* hot spot mutant *Candida krusei* isolates, 19 *FKS* wild-type *Candida parapsilosis* isolates, and 15 *FKS* hot spot wild-type and 4 *fks* hot spot mutant *Candida tropicalis* isolates. This collection of strains has been described and used in a previous work (1).

Quality control strains. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as quality control strains in all experiments.

Antifungal susceptibility testing. EUCAST microdilution methodology was performed by strictly following the EDef 7.1 standard guidelines (5). Plates were prepared in one batch, sealed in aluminum foil, and stored at −80°C for no longer than 2 months before use. Microtiter plates were read spectrophotometrically at 530 nm after 24 h, and the MIC was determined using 50% growth inhibition. One set of microplates contained a glucose concentration of 0.2%, whereas the other set contained the recommended glucose concentration of 2%.

Analysis of results. Statistical analysis was done using PASW statistics, version 18.0 (PASW, S.L., Madrid, Spain). MIC values were transformed to $\log_2$.

* Corresponding author. Mailing address: Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Ctra. Majadahonda-Pozuelo Km 2, 28220 Majadahonda, Spain. Phone: 34918223921. Fax: 34915097966. E-mail: jlrtudela@isciii.es.

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The reproducibility of the results obtained by reference techniques was calculated to determine the percentage of essential agreement (EA) between MIC values. Agreement was defined as discrepancies in MIC results of no more than ±1 2-fold dilution.

Linear regression analysis for both methods was done to test the linearity of the relationship between the MICs obtained with 2% of glucose versus those obtained with 0.2% glucose.

In addition, a two-way random-effect model was utilized to calculate the intraclass correlation coefficient (ICC) with a 95% confidence interval. The ICC is a reverse measurement of the variability of the counting values. The ICC was calculated using the formula ICC = (group mean square − error mean square)/(group mean square + error mean square) and thus has a maximum value of 1 if there is a perfect correlation and a minimum value of −1 if there is a complete absence of correlation. The ICC evaluates the correlation between values offering statistical significance since it takes into account the number of cases and absolute value of the counting. The ICC is the analysis which exhibits the highest statistical power for correlation studies.

Conclusions. The comparison between the MICs obtained with 0.2% glucose versus 2% glucose (EDef 7.1) showed that 61 strains (64.2%) had identical MICs, 32 (33.7%) had one 2-fold dilution difference (25 [78.1%] had one 2-fold dilution lower and 7 [21.8%] had one 2-fold dilution higher), and 2 (2.1%) had two 2-fold-dilution differences (one lower and one higher). The Pearson's coefficient obtained by means of linear regression was 0.913. The ICC using a random model of consistency for both variables, 2% glucose versus 0.2% glucose, was 0.954, with a 95% confidence interval of 0.931 to 0.969. When the same model was stressed with an absolute agreement model, the ICC was 0.950, with a 95% confidence interval of 0.922 to 0.968. Thus, statistical analysis shows that glucose concentration does not have a significant influence on the MIC values. MICs were essentially the same for 93 strains (97.8%) because the MICs were identical or had one 2-fold dilution difference. If the usual definition of essential agreement had been used (±2 2-fold dilutions), 100% agreement would have been obtained. ICC values obtained by means of essential agreement or absolute consistency were very close—0.954 versus 0.950—with very narrow 95% confidence intervals.

In summary, the Cancidas product insert includes a warning concerning the stability of caspofungin if dissolved in a glucose-containing solution. However, the concentration used in method EDef 7.1 does not influence the susceptibility values, as the MICs obtained with a glucose concentration of 0.2% (CLSI) of glucose were essentially the same as those obtained with a 2% (EUCAST) concentration. Alternative factors must account for the variability observed for differential MIC results obtained using EUCAST and CLSI susceptibility testing procedures.

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