

## Letter to Editor

# **Saccharomyces cerevisiae versus Candida in the Liofilchem® A.F. Genital System**

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A.F. Genital System (GS - ref. 74156; Liofilchem®, Roseto degli Abruzzi, Teramo, Italy) is a 24-well plastic tray (**Figure 1**) that provides a quick, presumptive identification of urogenital pathogens (from vaginal/urethral swabs and seminal fluid). Each well is inoculated with a suspension of the clinical specimen (in 3 mL of sterile physiological solution), then the panel is incubated at 36±1°C for 18-24 hours. The tray contains desiccated biochemical and antibiotic substrates and tests are interpreted based on color change of wells. According to the manufacturer's instructions, the system only detects *Candida* species, among yeasts, based on color change of well 24 (green, no growth of *Candida*; turbid yellow, *Candida* growth) along with the observation of blastospores, hyphae and chlamydo spores in well 6 (that contains a liquid growth medium), after incubation.

While studying a vaginitis case (unpublished data) however, we obtained a GS positivity (wells for *Candida*), with a concomitant massive growth of *Saccharomyces cerevisiae* on the agar medium (yeast identification was provided by a D1 region sequencing). Accordingly, multipolar buds (that are almost pathognomonic of *S. cerevisiae*, but are not produced by *Candida*) were observed in well 6 (**Figure 2**), in the absence of hyphae, along with yellow colour of well 24 (indicating fungal growth, as mentioned above).

Hence, we thought that the GS panel could not only permit replication of *Candida* (as stated in

the product instructions provided by the manufacturer), but that of *S. cerevisiae*, too. We tested then the tray with 50 *S. cerevisiae* clinical strains collected in our laboratory, as well with *S. cerevisiae* ATCC9763, *S. cerevisiae* IHEM 25104 (that we have deposited into the BCCM/IHEM collection of biomedical fungi and yeasts, Bruxelles, Belgium), and *C. albicans* ATCC 90028 (as positive control).

Fresh cultures (24h-incubation on Sabouraud agar, in air, at 30°C) were used. Well isolated colonies were emulsified in 3 mL of physiological sterile solution (provided by the manufacturer) to obtain suspensions with a final opacity of 0.5 MacFarland. These were inoculated in wells 6 and 24 (0.2 mL per well) of individual panels; wells 24 were covered with 1 drop of Vaseline Oil (provided by the manufacturer); trays were then covered with their plastic lid and incubated at 36°C, aerobically.

After 24 hours of incubation, wells 24 were observed to be yellow-coloured, while microscopic examination (40x without staining) of aliquots from wells 6 showed the formation of blastospores, unipolar buds and hyphae by *C. albicans* ATCC 90028; instead, blastospores, multipolar buds (**Figure 2**) and rare pseudohyphae had been produced by the 52 *S. cerevisiae* strains.

In the light of this, we can assess that GS is designed to detect *Candida* (among yeasts), but positivity may be due to *S. cerevisiae* (that is able to grow in both well 6 and well 24 of the



**Figure 1.** A.F. Genital System tray.

panel). Hence, it is important that operators who use this test as the only diagnostic approach to genital mycoses (without confirming positivity through cultures and fungal identification) be aware of this finding; particularly, observation of yellow color in well 24 must be followed by a careful examination of aliquots from well 6, with the observation of hyphae and unipolar buds suggesting the growth of *Candida*; conversely, the recovering of multipolar budding images (**Figure 2**) in the absence of hyphae will presumptively indicate the presence of *S. cerevisiae*.

From a clinical point of view, in fact, it is important to distinguish these two species each other, as *S. cerevisiae* is inherently less susceptible to azoles, while it may respond to other compounds (i. e. nystatin) [1-3].

Again, from an epidemiological point of view, misidentification of *S. cerevisiae* as *Candida*

may provide confused informations about the epidemiology of fungal agents of genital infections.

Actually, although most vulvovaginal mycoses are due to *Candida* (especially *C. albicans*), those caused by *S. cerevisiae* are emerging, perhaps owing to the wide use of fluconazole and itraconazole, to which the organism may be intrinsically less susceptible [1-3]. In this context, GS can provide treating physicians and patients with correct, although presumptive, yeast characterizations.

To conclude, there are no published works focusing on GS performance and, when possible, fungal identification provided by this system should be confirmed through cultures. However, laboratories that use it as the only diagnostic approach to genital infections may benefit from the fact that *S. cerevisiae* can be grown; so, unipolar and multipolar buds, along



**Figure 2.** *S. cerevisiae* multipolar budding (observed in well 6).

with hyphae and pseudohyphae observed in well 6 must be accurately recognized [4].

In our opinion, as soon as an exceedingly wider number of *S. cerevisiae* isolates are experimentally screened and detected by this system, the manufacturer could take into consideration to include this fungal species in the list of detectable pathogens.

#### **Conflict of interest statement**

None to declare.

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