Simple, Inexpensive, Reliable Method for Differentiation of
Candida dubliniensis from Candida albicans

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Candida dubliniensis is a recently described pathogenic species which shares many phenotypic features with
Candida albicans, including the ability to form germ tubes and chlamydospores. These similarities have caused
significant problems in the identification of C. dubliniensis by the average clinical mycology laboratory. To
facilitate the differentiation of these species, we investigated the growth of 120 isolates of C. dubliniensis and 98
C. albicans isolates at 42 and 45°C on Emmons' modified Sabouraud glucose agar (SGA) and 10 isolates of each
species in yeast-peptone-dextrose broth. None of the C. dubliniensis isolates grew on the agar or in the broth
medium at 45°C, while 11 isolates were capable of growing on SGA at 42°C. In contrast, all of the C. albicans
isolates but one grew at 45°C on or in either medium. These reproducible results clearly demonstrate that the
incubation of isolates suspected to be C. dubliniensis or C. albicans at 45°C provides a simple, reliable, and
inexpensive method for the differentiation of the two species.

Candida dubliniensis is a newly described species of the most
protean genus of pathogenic yeasts (9, 11). The vast majority of
C. dubliniensis isolates identified to date have been recovered
from the oral cavities of human immunodeficiency virus (HIV)-
infected individuals, particularly those suffering from recurrent
episodes of oral candidiasis (3, 8–11). However, a small num-
ber of isolates associated with other anatomical sites, including
the vagina and the lung, have been reported (5, 9, 11). To gain
a more complete understanding of the precise epidemiological
role that this species plays in human infections, it is essential
that rapid and reliable tests for the identification of C. dublini-
ensis in the routine clinical microbiology laboratory be avail-
able. However, the introduction of such tests has been com-
licated by the fact that C. dubliniensis shares many phenotypic
characteristics with Candida albicans (9). The high degree of
similarity between these two species has, in all likelihood, con-
tributed to the misidentification of some C. dubliniensis isolates
classified as C. albicans (3). The most reliable tests currently used for dis-
criminating between the two species are based on molecular tech-
niques such as DNA fingerprinting with repetitive-sequence-
containing DNA probes, pulsed-field gel electrophoresis, and
others (2, 9, 11). Although they are very effective, these tech-
niques are not readily applicable to the identification of large
numbers of isolates, nor can they presently be conducted in
most standard mycology laboratories. While the use of a num-
ber of phenotypic characteristics, e.g., chlamydospore produc-
tion, carbohydrate assimilation, colonial coloration on differ-
ential media such as CHROMagar Candida and methyl blue-
Sabouraud agar, has been investigated, none has provided a
completely reliable method for the differentiation of C. albi-
cans from C. dubliniensis (7, 9). It has previously been sug-
gested that the two species could be distinguished by the inhibi-
tion of the growth of C. dubliniensis at 42°C (2, 9, 11). How-
ever, one recent study reported that approximately 10% of C. dubliniensis isolates were able to grow, albeit poorly, at
this elevated temperature (9). Furthermore, another recent
study reported that 8 of 12 C. dubliniensis isolates examined
grew at 42°C (7). These findings motivated us to investigate
whether incubation at an even higher temperature would pro-
vide a simple, inexpensive, and reliable means of differentiat-
ing the two species.

Descriptions of the 120 C. dubliniensis isolates and 98 iso-
lates of C. albicans used in these studies are presented in Table
1. Conventional morphological and physiological methods as
well as molecular techniques were employed to identify iso-
lates of both species (11). All yeasts studied were initially
grown for 48 h at 37°C individually on 25 ml of Emmons’
modified Sabouraud glucose agar (SGA) contained in 85-mm-
diameter petri dishes. A small portion of a single colony of
each isolate was then aseptically removed and streaked over
the surface of two plates of SGA, one of which was incubated
at 42°C and the second of which was placed at 45°C. Growth,
if any, on both plates was visually assessed after 24 and 48 h of
incubation. In addition to these experiments, 10 isolates of
each species were selected for examination of their growth in
yeast-peptone-dextrose (YPD) broth at 37, 42, and 45°C. The
10 C. dubliniensis isolates included 3 which grew poorly at 42°C
on SGA medium and 7 that did not grow at all on SGA at that
temperature. Nine of the C. albicans isolates were randomly
selected, while the 10th isolate was chosen because it failed to
grow at 45°C on SGA (see below). By using a hemocytometer,
a standard inoculum suspension, containing 10⁶ CFU in sterile
distilled water, was prepared from colonies of each isolate
grown on SGA for 24 h at 37°C. The inoculum was then
aseptically transferred to YPD broth (to a final volume of 50
ml) in 250-ml conical flasks and incubated at the three tem-
peratures with shaking at 150 rpm. At specific time points,
aliquots of each isolate were removed to spectrophotometri-
cally measure their optical densities at 600 nm. These values
were then used to plot a growth curve for each isolate at each
temperature.

No growth was found for any of the 120 C. dubliniensis iso-
lates at 24 and 48 h on SGA at 45°C, although 11 isolates
YPD broth at 37 and 45°C are presented in Fig. 1. Although all representative growth curves for isolates of each species grown in YPD broth at 37°C (solid lines) and CD36 and CD43 in YPD broth medium at 37°C (dashed lines). O.D. 600, optical density at 600 nm.

*Each isolate was recovered from a separate individual.

These included two fecal isolates and one each from a blood culture, tracheal aspirate, sputum, and postmortem lung specimens.

<table>
<thead>
<tr>
<th>Species and country of isolation</th>
<th>No. of isolates</th>
<th>Specimen source(s)</th>
<th>HIV status of subject(s)</th>
<th>Source or reference(s)</th>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Argentina</td>
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<td>+</td>
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</tr>
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</tr>
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<tr>
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</table>

[Table 1. C. dubliniensis and C. albicans isolates used in these studies]

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