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Short Report

Evaluation of a Newly Developed Identification Kit, RID Zyme CAS Test, for *Candida albicans*

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Abstract

To evaluate a newly developed identification kit, the RID Zyme CAS test for *Candida albicans*, 1136 *C. albicans* and 403 non-*albicans Candida* strains were tested. Distinction of medically important non-*albicans* strains, with the exception of *C. dubliniensis*, was obtained. These results show that this new kit is simple and effective for the identification of *C. albicans* in clinical samples. Furthermore, the one hour period for identification makes it very attractive.

Key words: *Candida albicans*, newly developed identification kit, RID Zyme CAS test

Candida albicans is the most frequently isolated yeast pathogen, and candidiasis is increasingly a complication in both immunocompromised and immunocompetent individuals. Immediate identification of the pathogen is usually required, however, conventional methods such as the API 20C AUX and ID32C systems (bioMerieux Japan, Tokyo, Japan) take more than 24 to 48 hours to give results. Several researchers have attempted to develop rapid methods for the identification of *C. albicans*. Perry *et al.*[1] and Dalton *et al.*[2] reported rapid methods that make use of the fluorogenic substrate (4-methylumbelliferyl-*N*-acetyl-β-D-galactosaminide) of β-galactosaminidase (EC 3.2.1.30). Perry *et al.*[1] reported a modification of their previous method, which involves alteration of the test substrate and inclusion of a second substrate in one reaction tube and provides a colorimetric rather than a fluorometric reaction product[3].

Here, we report the evaluation of a newly developed identification kit, the RID Zyme CAS test, on 1136 *C. albicans* and 403 non-*albicans Candida* species and five serotypes of *Cryptococcus neoformans*.

Strains used in this study are listed in Tables 1 and 2. These strains are registered at the Research Center for Pathogenic Fungi and Microbial Toxicoses of Chiba University and were cultured on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) at 25°C for 48 hours. Most of the strains were previously identified using the ID32C system (bioMerieux Japan) and/or the Candida Check system (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan), and were genotyped by PCR of 25S rDNA[4] and the topoisomerase II gene[5]. Some strains were purchased from ATCC: American Type Culture Collection (Manassas, VA, USA), CBS: Centraalbureau voor Shimmelcultures, (Delft, the Netherlands), IFO: Institute for Fermentation, Osaka (Osaka, Japan) or NBRC: National Institute of Technology and Evaluation Biological Resource Center (Chiba, Japan) as references.

The RID Zyme CAS test (Mitsubishi Kagaku Iatron, Inc.) was put out to the market in 2004. Although two of the enzymes *C. albicans* has are β-galactosaminidase and L-proline aminopeptidase, those substrates (MNGL, 4-methylumbelliferyl-*N*-acetyl-β-D-galactosaminide for β-galactosaminidase; fluorogenic and PRO, L-proline *p*-nitroanilide for L-proline aminopeptidase; chromogenic) are infused on a cotton swab in this kit. After picking up a single colony with the swab, one can determine within approximately 1 hour whether the isolate is *Candida albicans*. The procedure is very simple: pick-up of one colony with a swab, incubation for 1 hour at 37°C with one drop of accompanying buffer, then visualization with a UV lamp (366 nm) and addition of a color

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Table 1. Specificity of RID Zyme CAS test

Organism	No. tested	MNGL	PRO
Candida albicans (serotypes A, B)	1136	+	+
C. dubliniensis	21	+	+
C. stellatoidea	5	+	−
C. glabrata	54	−	−
C. guilliermondii	15	−	+
C. kefyr	4	−	−
C. krusei	20	−	−
C. parapsilosis	138	−	+
C. tropicalis	107	+	−
Cryptococcus neoformans (serotypes A, B, C)	3	−	−
Cr. neoformans (serotypes D, AD)	2	+	−

MNGL, 4-methylumbelliferyl-*N*-acetyl- β-D-galactosaminide; PRO, L-proline *p*-nitroanilide.
+, positive; -, negative.

Table 2. Non-*albicans Candida* species tested

Species distinguishable from *C. albicans* by RID Zyme CAS test

Clinically important species

- *glabrata* (CBS 138 T and 53 isolates)
- *guilliermondii* (ATCC 22995 T and 14 isolates)
- *kefyr* (ATCC 4135 T and 3 isolates)
- *krusei* (IFO 1395 T and 19 isolates)
- *parapsilosis* (IFM 5751 and 137 isolates)
- *stellatoidea* (ATCC 11006 T and 4 isolates)
- *tropicalis* (IFM 41420 and 106 isolates)

Others

- *apicola* (ATCC 24616 T)
- *boleticola* (CBS 6420 T)
- *bombi* (ATCC 18811 T)
- *chilensis* (ATCC 22076 T)
- *chiropterorum* (ATCC 22291 T)
- *cylindracea* (ATCC 14830 T)
- *famata* (IFM 47882)
- *haemulonii* (ATCC 22991 T)
- *holmii* (IFO 1128 T)
- *inconspicua* (CBS 180 T)
- *intermedia* (ATCC 14439 T)
- *lipolytica* (IFM 5475)
- *lusitaniae* (IFM 49723)
- *magnoliae* (CBS 166 T)
- *melibiosica* (IFO 10238 T)
- *melinii* (IFM 5473)
- *mesenterica* (IFO 1123 T)
- *mogii* (IFO 0436 T)
- *molischiana* (IFO 10296 T)
- *norvegensis* (CBS 1922 T)
- *oleophila* (IFO 1021 T)
- *pelliculosa* (IFM 47124)
- *saitoana* (ATCC 36584 T)
- *santamariae* (IFO 1982 T)
- *santamariae* var. *membranifaciens* (CBS 5838 T)
- *silvatica* (IFO 10311 T)
- *sphaerica* (IFM 48789)
- *torresii* (IFO 10421 T)
- *tsuchiyae* (IFO 10167 T)
- *utilis* (IFM 40125)
- *viswanathii* (CBS 4024 T)
- *zeylanoides* (IFO 1663 T)

Species indistinguishable from *C. albicans* by RID Zyme CAS test

- *kruisii* * (ATCC 24408 T)
- *sake* * (NBRC 1354)
- *savonica* * (IFO 10309 T)
- *suecica* * (IFO 10313 T)
- *catenulata* (ATCC 10565 T)
- *dubliniensis* (CBS 7987 T and 20 isolates)
- *maltosa* (IFM 52017)
- *rugosa* (CBS 613 T)

*, No growth at 37°C; T, type strain.

development reagent (*p*-dimethylaminocinnamaldehyde). If the isolate is *C. albicans*, MNGL fluorescence is produced, and the PRO chromogen turns the swab purple (Fig. 1).

Results of the RID Zyme CAS test with nine clinically important *Candida* species and two *Cr. neoformans* species are shown in Table 1. All of the 1136 strains of *C. albicans* tested, including

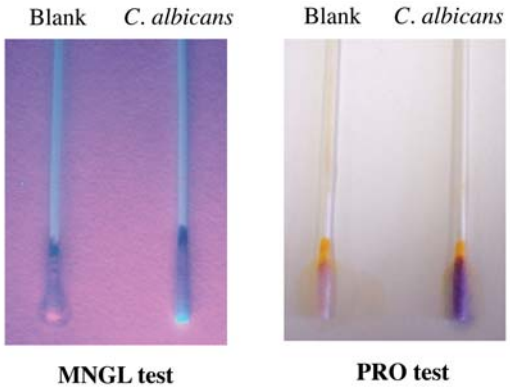

Fig. 1 Results of RID Zyme CAS test.

MNGL, 4-methylumbelliferyl-*N*-acetyl- β-D-galactosaminide; PRO, L-proline *p*-nitroanilide.

serotype B strains, were positive in both tests (MNGL and PRO tests). A recently classified atypical *C. albicans* species, *C. dubliniensis*, was also positive in both tests. *C. stellatoidea*, about which the taxonomy difference with *C. albicans* has been argued[4, 6-8] and which now is recognized as a synonym of *C. albicans*, was only positive in the MNGL test. Clear distinction from *C. albicans* was obtained for *C. parapsilosis*, *C. guilliermondii*, *C. kefyr*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *Cr. neoformans*. With the exception of eight species (*C. catenulata*, *C. dubliniensis*, *C. kruisii*, *C. maltosa*, *C. rugosa*, *C. sake*, *C, savonica*, *C. suecica*), distinction from *C. albicans* was obtained with other non-*albicans* species (Table 2). Four of the eight indistinguishable species (*C. kruisii*, *C. sake*, *C. savonica*, *C. suecica*) showed no growth at 37°C, and four species (*C. catenulata*, *C. dubliniensis*, *C. maltosa*, *C. rugosa*) showed no distinction from *C. albicans* with the kit. However, *C. maltosa* has been reported as non-pathogenic for mice[9], and distinction of *C. catenulata* and *C. rugosa* from *C. albicans* can be made microscopically[10]. Summarizing these data, the sensitivity of this kit was 100% and the specificity was 97.6%. On the other hand, Crist *et al.*[11] and Heelan *et al.*[12] compared four methods (MUREX *C. albicans*, Albicans-Sure, BactiCard Candida and the germ tube test), when the operation time of BactiCard Candida and Albicans-Sure was being emphasized as 5 minutes. Although the operation time of the RIDzyme CAS test is 1 hour, it is superior to those two methods in that one colony is sufficient for the inoculation. The specificity of those four methods apparently was higher than the RIDzyme CAS test since *C. dubliniensis* was not taken into consideration. According to these results, the RID Zyme CAS test kit is effective in the identification of *C. albicans* from clinical samples. Unfortunately, *C. dubliniensis*, which has been increasingly reported in recent years, was indistinguishable from *C. albicans*.

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