Application of Agglutinins for the Rapid and Accurate Identification of Medically Important Candida Species

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Received for publication 13 January 1970

Agglutinins have been prepared against the medically important Candida species. Crude antisera to the various species demonstrated intense cross-reactions with heterologous yeastlike fungi as well as with many true yeasts. However, carefully monitored adsorptions of selected antisera allowed the production of six factor sera that proved useful in a slide agglutination test. These six sera permitted the rapid and specific identification of C. guilliermondii, C. krusei, C. parapsilosis, and C. pseudotropicalis. They also allowed the delineation of two groups: (i) C. albicans (type A)-C. tropicalis and (ii) C. albicans (type B)-C. stellatoidea. C. albicans type A could be readily distinguished from C. tropicalis by its ability to form germ tubes in serum. C. stellatoidea could be distinguished from C. albicans type B by its predominantly filamentous growth on a nutritionally deficient medium. The medically important Candida species could be identified within 24 hr by the combined use of serological and morphological procedures.

The possibility of using agglutination procedures to identify Candida species has been explored extensively by several investigators (9, 12, 13, 14). In a comprehensive study, Tsuchiya et al. (14) carried out antigenic analyses on members of the genus and reported the successful production and application of monospecific agglutinins. They recommended the use of these reagents in a slide agglutination test for the rapid identification of Candida species.

However, neither the agglutination test of Tsuchiya et al. nor any other serological procedure for identification of the Candida species has been adopted for routine use in the diagnostic laboratory. This may be attributed in part to the fact that many laboratories are not aware of the importance of identifying Candida species other than C. albicans. Also difficulties have been encountered in the production of monospecific serological reagents (12). This problem has been partially explained by the discovery of two serotypes of C. albicans that are not only antigenically closely related to each other but also to other Candida species (4, 7).

The objective of the present investigation was to develop and prepare reproducible monospecific antisera applicable to the rapid and accurate identification of the medically important Candida species. If this goal could not be attained, then the purpose was to use the reagents obtained, in such combinations, so that seroidentity of these Candida species could be accomplished.

MATERIALS AND METHODS

Cultures. C. albicans ATCC 10259, C. albicans G62, C. guilliermondii ATCC 9058, C. krusei J-1005, C. parapsilosis ATCC 10232, C. pseudotropicalis J-1004, C. stellatoidea 44, and C. tropicalis ATCC 7349 were used in preparing immunogens and agglutinogens. The identity of these and 198 other isolates of Candida, as well as that of the noncandidal yeastlike fungi used in this study, was verified by procedures recommended by Ajello et al. (1).

Antigen preparation. Antigens were prepared from 48-hr cultures grown in 500 ml of dextrose-peptone-yeast extract broth (dextrose, 2%; peptone, 1%; and yeast extract, 0.5%) at 25 C. During incubation, cultures were rotated at 130 rev/min on a gyratory shaker. After incubation, all cultures were formalinized (0.5%) and subjected to appropriate sterility and purity checks. The cells were then harvested, washed three times, and suspended in 0.9% NaCl solution containing 0.5% Formalin.

Antisera production. Two to four rabbits, without detectable Candida agglutinins, were injected intra-

1 A portion of a dissertation submitted by the first author to the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Public Health in the School of Public Health.

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venously with a 1.0-ml volume of increasing concentration of each antigen. Immunization was continued on alternate days for a period of 2 weeks, until a total inoculum was attained for each animal of approximately 8.5 × 10⁶ cells. One week after receiving the final injection, the rabbits were exsanguinated by cardiac puncture. Antisera to the same antigen, demonstrating similar titers, were pooled, preserved with Merthiolate (1:10,000), and stored at −20 C.

Agglutination procedures. Agglutinin titers with homologous and heterologous antigens were determined by the tube agglutination test. Antigens were adjusted to a concentration of 3 × 10⁶ cells per ml with a hemacytometer. Twofold dilutions of the serum in 0.25-ml volumes were mixed with an equal volume of the standardized cell suspension. Titrations were read after 2 hr of incubation at 37 C and after overnight refrigeration at 4 C.

Once agglutination patterns were established, the slide agglutination test was used. A drop of serum was placed in a quarter-section marked on a microscope slide [1 by 3 inches (2.54 by 7.62 cm)], mixed with a similar quantity of formalinized antigen, turbidometrically adjusted to a no. 10 McFarland nephelometer standard. The degree of clumping was read after 30 sec. Agglutinations of 2 + or greater were recorded as positive.

Adsorption procedures. Antisera were adsorbed with half-volumes of packed and drained cells at 25 C for 1 min. Adsorptions were carried out until no reaction occurred with the adsorbing antigen.

Morphological tests. Two morphological tests were used in this study, the serum-germ tube test (8) and the Kamaya test (6). The former procedure was performed by incubating the test organism into bovine serum and incubating at 37 C for 2 to 3 hr. The production of germ tubes within 3 hr by yeastlike cells was considered positive for the identification of C. albicans.

The Kamaya test was performed by inoculating a 1:10 diluted Sabouraud broth with the test organism and incubating at 37 C for 1 hr. The surface of a corn meal-agar (without added dextrose) plate was swabbed with this cell suspension, and the culture was incubated at 37 C for 24 hr. The detection of yeast cells associated with true mycelium was considered positive for the identification of C. stellatoidea.

RESULTS

Serological response in rabbits. Homologous and heterologous agglutinin titers of the unadsorbed antisera produced against each of the seven species of Candida were determined. Homologous titers of 1:1,024 to 1:2,048 were consistently obtained. However, no antiserum was monospecific, and the homologous titers were not significantly higher than those obtained with the heterologous antigens. Consequently, it was necessary to use adsorption procedures to render the antisera specific for the antigen-antibody reactions desired.

Adsorption patterns. Cross-adsorption of all of the Candida sera indicated that at least 13 different agglutination patterns were demonstrable.

The 13 patterns of reaction and the combinations of antisera and adsorbing antigens responsible for them are listed in Table 1. Each of these distinct serum reactions was arbitrarily assigned a number. Antisera demonstrating each of the patterns were identified with each of these reaction pattern numbers and referred to as factor sera. By using these data, antigenic relations among the Candida species studied were determined and the scheme shown in Table 2 was established. It is evident from the data presented that species-specific antibody could be produced against C. guillermondii, C. krusei, C. parapsilosis, and C. pseudotropicalis. Specific antibodies could not be produced for C. albicans, C. stellatoidea, or C. tropicalis. However, these three species could be differentiated from the other Candida species.

Dependability of reagents. The 13 factor sera were stable for more than 1 year when stored at 4 C or at −20 C. Reproducibility, sensitivity, and specificity were determined by repeatedly testing each factor serum in blind runs against 112 well-identified isolates of the seven species of Candida. These isolates are listed in Table 4. Reproducibility was a measure of the capacity of each reagent to perform in an identical manner when tested repeatedly against the same antigen. Sensitivity was a measure of the capacity of the reagents to detect an antigen when present, and specificity was the measure of its failure to react with heterologous antigens.

The data in Table 3 reveal that factor sera 1 through 7 were highly reproducible and demonstrated sensitivities and specificities that were greater than 90%. The remaining factor sera were unsatisfactory reagents, because of poor reproducibility (factor serum 12), poor sensitivity (factor sera 8, 9, and 12), or poor specificity (factor sera 10, 11, and 13). It is apparent (Fig. 1) that the most reliable reagents (factor sera 2 through 7) enabled identification of C. guillermondii, C. krusei, C. parapsilosis, and C. pseudotropicalis. The remaining three species could be placed into two groups: (i) C. albicans A-C. tropicalis and (ii) C. albicans B-C. stellatoidea. Serological differentiation of these four fungi was not possible; therefore, two morphological tests were used in conjunction with the serological tests. C. albicans A can be differentiated from C. tropicalis by the ability of the former to produce germ tubes in serum (8). C. stellatoidea can be differentiated from C. albicans B by the ability of the former to produce extensive true mycelium when tested by the method of Kamaya (6).

The 112 Candida isolates used to evaluate the factor sera were coded and subjected to analysis by the scheme shown in Fig. 1. All of these fungi were accurately identified by using factor sera
| Factor no. | Antiserum to Candida | Adsorbed with Candida | Reaction with Candida |
|------------|----------------------|-----------------------|-----------------------|
|            |                      | homologous            | albicans A | albicans B | guilliermondii | kruusei | parapsilosis | pseudotropicalis | stellatoidea | tropicalis |
| 0          | all species          | homologous            | -         | -         | -             | -       | -           | -               | -           | -         |
|            | normal serum         | albicans A, guilliermondii, stellatoidea | -         | -         | -             | -       | -           | -               | -           | -         |
|            | albicans B           | albicans A, albicans B, guilliermondii | -         | -         | -             | -       | -           | -               | -           | -         |
|            | stellatoidea         | albicans A            | -         | -         | -             | -       | -           | -               | -           | -         |
|            | tropicalis           | albicans A            | -         | -         | -             | -       | -           | -               | -           | -         |
| 1          | all species          | none                  | +         | +         | +             | +       | +           | +               | +           | +         |
| 2          | albicans B           | kruusei, pseudotropicalis | +         | +         | +             | +       | -           | -               | +           | +         |
|            | parapsilosis         | kruusei, pseudotropicalis | +         | +         | -             | +       | -           | -               | +           | +         |
|            | stellatoidea         | kruusei, pseudotropicalis | +         | +         | -             | +       | -           | -               | +           | +         |
| 3          | albicans A           | albicans B, stellatoidea | +         | -         | +             | -       | -           | -               | -           | +         |
|            | tropicalis           | albicans B, stellatoidea | +         | -         | +             | -       | -           | -               | -           | +         |
| 4          | albicans A           | guilliermondii        | -         | -         | -             | -       | -           | -               | -           | -         |
|            | tropicalis           | guilliermondii        | -         | -         | -             | -       | -           | -               | -           | -         |
| 5          | albicans A           | kruusei, parapsilosis, pseudotropicalis | -         | +         | +             | -       | -           | -               | +           | +         |
|            | albicans B           | kruusei, parapsilosis | -         | +         | +             | -       | -           | -               | +           | +         |
|            | guilliermondii      | kruusei, parapsilosis | -         | +         | -             | -       | -           | -               | +           | +         |
|            | stellatoidea        | kruusei, parapsilosis | -         | +         | -             | -       | -           | -               | +           | +         |
|            | tropicalis           | kruusei, parapsilosis | -         | +         | -             | -       | -           | -               | +           | +         |
|            |                       |                      | -         | -         | -             | -       | -           | -               | -           | -         |
| 6          | kruusei              | albicans A, albicans B, guilliermondii, parapsilosis, pseudotropicalis, stellatoidea, tropicalis | -         | -         | -             | +       | -           | -               | -           | -         |
| 7          | pseudotropicalis     | albicans A, albicans B, guilliermondii, kruusei, parapsilosis, stellatoidea, tropicalis | -         | -         | -             | -       | +           | -               | -           | -         |
| 8          | parapsilosis         | albicans A, albicans B, guilliermondii, stellatoidea | -         | -         | -             | -       | +           | -               | -           | -         |
| 9          | guilliermondii       | albicans A, albicans B, stellatoidea, tropicalis | -         | -         | +             | -       | -           | -               | -           | -         |
| 10         | albicans A           | tropicalis           | +         | +         | -             | +       | -           | +               | -           | -         |
|            | parapsilosis         | tropicalis           | +         | +         | -             | +       | -           | +               | -           | -         |
| 11         | guilliermondii       | pseudotropicalis     | +         | +         | +             | +       | -           | +               | -           | -         |
|            | parapsilosis         | pseudotropicalis     | +         | +         | +             | +       | -           | +               | -           | -         |
| 12         | albicans B           | tropicalis           | -         | +         | -             | -       | +           | +               | -           | -         |
|            | stellatoidea         | tropicalis           | -         | +         | -             | -       | +           | +               | -           | -         |
| 13         | parapsilosis (172)   | albicans A (B311)    | -         | +         | -             | +       | -           | -               | -           | -         |
TABLE 2. Antigenic composition of the medically important species of Candida

| Species               | Antigens recognized |
|-----------------------|---------------------|
| C. albicans A......... | 1 2 3 4 5 10 11     |
| C. albicans B......... | 1 2 5 10 11 12 13   |
| C. guilliermondii..... | 1 2 3 5 9 10 11     |
| C. krusei ............. | 1 6 11              |
| C. parapsilosis....... | 1 2 8 10 11 12 13   |
| C. pseudotropicalis... | 1 7                |
| C. stellatoidea....... | 1 2 5 10 11         |
| C. tropicalis......... | 1 2 3 4 5 11        |

TABLE 3. Evaluation of the 13 factor sera as to reproducibility, sensitivity, and specificity in reaction with 112 known isolates of medically important Candida species

| Factor serum | No. of times factor serum used | Per cent | | | |
|--------------|-------------------------------|----------|---|---|---|
|              |                               | Reproducibility | Sensitivity | Specificity |
| 1            | 322                           | 100       | 99 | 98 |
| 2            | 440                           | 100       | 98 | 98 |
| 3            | 380                           | 99        | 96 | 97 |
| 4            | 377                           | 98        | 98 | 91 |
| 5            | 244                           | 100       | 97 | 97 |
| 6            | 119                           | 100       | 97 | 94 |
| 7            | 102                           | 100       | 100| 94 |
| 8            | 130                           | 91        | 57 | 98 |
| 9            | 105                           | 93        | 54 | 100|
| 10           | 229                           | 96        | 93 | 97 |
| 11           | 91                            | 98        | 92 | 70 |
| 12           | 72                            | 78        | 43 | 94 |
| 13           | 136                           | 97        | 91 | 70 |

TABLE 4. Identification of coded cultures of the medically important Candida species by using six factor sera and two morphological tests

| Species                  | No. of isolates | No. Identified |
|--------------------------|-----------------|---------------|
| C. albicans A............ | 29              | 29 0 100      |
| C. albicans B............ | 13              | 13 0 100      |
| C. guilliermondii....... | 12              | 12 0 100      |
| C. krusei ............... | 11              | 11 0 100      |
| C. parapsilosis.......... | 15              | 14 1 93       |
| C. pseudotropicalis...... | 7               | 7 0 100       |
| C. stellatoidea.......... | 8               | 8 0 100       |
| C. tropicalis............ | 17              | 17 0 100      |

and the two morphological tests, except 1 of the 15 isolates of C. parapsilosis (Table 4). In addition, 38 yeasts and yeastlike isolates representing six genera were examined to determine the specificity of the factor sera with medically unimportant Candida species and other heterologous fungi. In no case did the factor sera agglutinate 11 Cryptococcus, 3 Rhodotorula, 4 Saccharomyces, and 3 Trichosporon species tested. However, 1 of 3 isolates (C. macedoniensis) representing 3 medically unimportant Candida species and 1 of 14 Torulopsis glabrata isolates agglutinated in the reagents.

Survey of diagnostic cultures. Forty-eight cultures of yeastlike fungi that were isolated from clinical material and submitted to the Mycology

![Flow chart for the identification of the medically important Candida species by using six selected factor sera.](image)
TABLE 5. Comparison of conventional and rapid serological procedures in the identification of yeasts and yeastlike fungi isolated from clinical material

| Fungus identity (conventional procedures) | No. of isolates | Pseudomycelium | Agglutination reaction | Fungus identity (serological procedures) |
|------------------------------------------|-----------------|----------------|------------------------|------------------------------------------|
| *Aureobasidium* species                   | 1               | -              | +                      | *Candida albicans* A (16)                  |
| *Candida albicans*                       | 18              | +              | +                      | *C. albicans* B (2)                        |
| *C. guilliermondii*                      | 1               | +              | +                      | *C. guilliermondii*                        |
| *C. natalensis*                          | 2               | +              | +                      | *C. tropicalis*                            |
| *C. parapsilosis*                        | 5               | +              | +                      | *C. parapsilosis*                          |
| *C. tropicalis*                          | 3               | +              | +                      | *C. tropicalis*                            |
| *Cryptococcus* species                   | 6               | -              | -                      | NT                                        |
| *Debaryomyces* species                   | 1               | -              | -                      | NT                                        |
| *Endomycopsis* species                   | 1               | +              | -                      | Not *Candida*                              |
| *Rhodotorula* species                    | 8               | -              | -                      | NT                                        |
| *Torulopsis* species                     | 1               | +              | -                      | Not *Candida*                              |

*Not tested with agglutinins because morphological studies indicated the fungus not to be a *Candida* species.

TABLE 6. Recommended methods for the production of factor sera

| Factor no. | Antiserum     | Adsorbing antigen     |
|------------|---------------|-----------------------|
| 2          | *C. albicans* B-G62 | *C. krusei* J-1005    |
| 3          | *C. albicans* A-10259 | *C. albicans* B-G62  |
| 4          | *C. albicans* A-10259 | *C. guilliermondii* 9058 |
| 5          | *C. albicans* A-10259 | *C. parapsilosis* 10232 |
| 6          | *C. krusei* J-1005 | *C. albicans* A-10259 |
| 7          | *C. pseudotropicalis* J-1004 | *C. albicans* A-10259 |

Section, National Communicable Disease Center, for identification were tested in parallel by routine methods and by the slide agglutination-morphology test scheme described above. In all cases, the true identity of each isolate was based upon the results obtained by the diagnostic laboratory. All cultures were first inoculated onto corn meal-agar (without added dextrose) to determine their ability to produce pseudomycelium. The pseudomycelium-producing cultures were then subjected to analysis with the factor sera and, if necessary, examined by the serum-germ tube test and the Kamaya test.

Pseudomycelium-producing yeasts that agglutinated in the antisera were always *Candida* species (Table 5). When serological and morphological criteria were used, 96% of the unknowns were correctly identified if they were medically important *Candida* species, or properly eliminated from consideration if they were not. One uncommonly encountered species of *Candida*, *C. natalensis*, was misidentified as *C. tropicalis* by the serological procedure. All unknowns, but the two *C. albicans* B isolates, were processed within 3 hr of receipt. Identification of the *C. albicans* B isolates required 24 hr because of the need of morphological characterization by the Kamaya test.

**Production of factor sera.** The recommended factor sera could be produced by a number of different combinations of antisera and adsorbing cells. The most effective methods for producing diagnostically useful factor sera are shown in Table 6. Four antisera and five adsorbing strains were used to produce all of the reagents necessary for the proper identification of the medically important *Candida* species.

**DISCUSSION**

Our study, in accord with others (5, 10, 11, 13), revealed a strong degree of antigenic unity among the various species of *Candida*. Nevertheless, distinct antigens for *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *C. pseudotropicalis* were recognized, and specific antibodies to them were prepared by use of appropriate adsorbing procedures (Table 1). In addition, nine other factor sera were prepared against antigens that are shared by two or more of the species studied. The production and reactivity of these reagents agree essentially with the findings of Tsuchiya et al. (14). However, we made no attempt to delineate reactions as to the involvement of heat-labile or heat-stable antigens, and, consequently, our coding is not the same as theirs.

The data revealed that all four of the mono-
specific reagents are stable and reproducible (Table 3), but two factor sera (8 and 9), the C. parapsilosis and C. guilliermondii sera, respectively, had low sensitivities and could not be used in a diagnostic scheme. Of the remaining nine factor sera, four were not useful because of poor specificity (factor sera 10, 11, and 13) and poor sensitivity and reproducibility (factor serum 12). Because factor serum 1 did not lead to any separation among the species, it was not useful in an identification scheme. Therefore, only six factor sera could be useful to identify the seven medically important Candida species.

Contrary to the results of Tsuchiya et al. (14) and Murray and Buckley (9), neither monospecific nor other antibody reagents could be produced to differentiate C. albicans from C. tropicalis and C. stellatoidea. Qualitative differences among these fungi were suggested by adsorption studies (Table 1, factor sera 10 and 13); however, further investigation with large numbers of isolates did not substantiate this and indicated that the differences were merely quantitative (Table 3).

The inability to differentiate the three important species, C. albicans, C. stellatoidea, and C. tropicalis, with serological reagents is a distinct disadvantage. This inability, however, in no way detracts from the value of the reagents that have been produced. Several physiological and morphological tests can be used in conjunction with serological procedures to delineate the antigenically indistinguishable groups; the serum-germ tube and the Kamaya tests, which are simple and rapid procedures, were found to be effective. Each of these tests functioned with 100% accuracy in this study (Table 4).

The antigenic closeness of many heterologous yeasts and yeastlike fungi is sufficiently established, so that the agglutination of Torulopsis isolates in selected factor sera was not surprising (3). This problem, however, was easily resolved by the use of corn meal-agar, which permitted the selection of only the pseudomycelium-producing yeastlike fungi for subsequent serological testing. Some isolates of Candida, such as C. guilliermondii, are slow to produce pseudomycelium. Therefore, corn meal-agar cultures must be incubated for at least 3 days before a fungus is considered not to be a Candida species and not to require serological testing because of the failure to produce pseudomycelium. Pseudomycelium-producers that were not Candida species (Endomycopsis species and Trichosporon species) presented no problem, because they were negative in the serological scheme.

Medically unimportant Candida species are occasionally recovered from clinical material and can present a diagnostic problem. These fungi produce pseudomycelium, and our data indicate that they would be selected from corn meal-agar plates for serological testing. Approximately 50% of these nonpathogenic Candida species are reactive with one or more of the selected factor sera. However, these saprophytic Candida species constitute less than 1% of all yeastlike fungi found in clinical material (2), and, because accuracy with the factor sera is comparable to that with conventional procedures (96%, Table 5), we do not consider the problem a formidable one.

We recommend that serological reagents be applied to unknown pure cultures of yeastlike organisms immediately after their receipt in the laboratory or after the isolation and selection of a pure yeastlike culture from clinical material. A corn meal-agar plate should be simultaneously inoculated. The interpretation of results of positive agglutinations must await evidence from the corn meal-agar culture that the fungus is a pseudomycelium producer. Fermentation and assimilation tests need not be carried out if the serological and morphological procedures demonstrate that an isolate is one of the medically important species of Candida.

ACKNOWLEDGMENT

This investigation was supported by the Laboratory Practice Training Program (Public Health Service training grant 2T01 GM 567 from the National Institute of General Medical Services).

LITERATURE CITED

1. Ajello, L., L. K. Georg, W. Kaplan, and L. Kaufman. 1963. Laboratory Manual for Medical Mycology. Public Health Service Pub. No. 994.
2. Bump, C. M., and L. J. Kunz. 1968. Routine identification of yeasts with the aid of molybdate-agar medium. Appl. Microbiol. 16:1503-1506.
3. Hasenclever, H. F., and W. O. Mitchell. 1960. Antigenic relationships of Torulopsis glabrata and seven species of the genus Candida. J. Bacteriol. 79:677-681.
4. Hasenclever, H. F., and W. O. Mitchell. 1961. Antigenic studies of Candida. I. Observation of two antigenic groups in Candida albicans. J. Bacteriol. 82:570-573.
5. Hasenclever, H. F., and W. O. Mitchell. 1964. Immunochemical studies on polysaccharides of yeasts. J. Immunol. 93:763-771.
6. Kamaya, T. 1968. Simple rapid identification of Candida albicans with emphasis on the differentiation between Candida albicans and Candida stellatoidea. Mycopathol. Mycol. Appl. 35:105-112.
7. Kaplan, W., and L. Kaufman. 1961. The application of fluorescent antibody techniques to medical mycology—a review. Sabouraudia 1:137-144.
8. Mackenzie, D. W. R. 1962. Serum tube identification of Candida albicans. J. Clin. Pathol. 15:563-565.
9. Murray, I. G., and H. R. Buckley. 1966. Serological study of Candida species. In H. I. Winner and R. Hurley (ed.), Symposium on Candida Infections. E. and S. Livington, Ltd., London.
10. Skinner, C. E., and D. W. Fletcher. 1960. A review of the genus Candida. Bacteriol. Rev. 24:397-416.

11. Summers, D. F., A. P. Grollman, and H. F. Hasenclever. 1963. Polysaccharide antigens of Candida cell wall. J. Immunol. 92:491-499.

12. Tsuchiya, T., Y. Fukazawa, and S. Kawakita. 1959. A method for the rapid identification of the genus Candida. Mycopathol. Mycol. Appl. 10:191-206.

13. Tsuchiya, T., Y. Fukazawa, and S. Kawakita. 1961. Serological classification of the genus Candida, p. 34-46. In Studies on candidiasis in Japan. Research Committee of Candidiasis, Education Ministry of Japan.

14. Tsuchiya, T., Y. Fukazawa, F. Miyasaki, and S. Kawakita. 1955. Studies on the classification of the genus Candida: thermostable and thermolabile antigens of the seven species of the genus Candida. Jap. J. Exp. Med. 25:75-83.