Pagano-Levin *Candida* Test Medium: Evaluation Using Vaginal Samples

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Since 1958 Pagano-Levin medium (PLM) has been used as a aid in the identification of *Candida albicans*. However, no statistical analysis of its effectiveness based on large number of random human specimens has been reported. The present study compared PLM (now called *Candida* test) with Sabouraud’s plus antimicrobials and Littman’s ox-gall agar without antimicrobials. Of 500 random vaginal samples 24.8 were true positives, 71.9% were true negatives, 2.2% were false positives, and 1.2% were false negatives on PLM. If only samples identified as *C. albicans* were considered, 95.4% were true positives and 4.6% were false negatives. PLM did not inhibit *C. albicans* from the vagina. No one medium was found superior to the others for the purpose of isolating and identifying *C. albicans*. Of the five strains of *C. parapsilosis*, the only other *Candida* species isolated, all the samples grew on PLM but most were inhibited on Sabouraud’s medium plus antimicrobials.

Pagano, Levin, and Trejo (30) described a medium formulated to aid in differentiation of *Candida albicans* from other species of the genus based on development of color upon growth of yeasts on a medium containing 2,3,5-triphenyltetrazolium chloride. *Candida* spp. varied in reduction of tetrazolium. Most *Candida* spp. formed colored formazans, but *C. albicans* either did so slightly or not at all. With the optimum concentration of 2,3,5-triphenyltetrazolium chloride was 100 μg/ml (0.01%), *C. albicans* produced creamy-textured, white to pink colonies. Other *Candida* spp. produced dark reds with a creamy texture or a white-chalky colony. The medium also included neomycin to suppress bacteria.

Though not cited by Pagano et al., Canizares and Shatin in 1951 (8) had used 2,3,5-triphenyltetrazolium chloride in dermatophyte culture media; 0.01% 2,3,5-triphenyltetrazolium chloride produced pink colonies of *C. albicans* with a white edge 14 days after inoculation.


Kutscher et al. (18), using American Type Culture Collection isolates, found it relatively accurate and confirmed the color variation for *C. albicans* colonies already described (8). Occasional colonies had a white border and pigmented center. Rosenthal and Furnari (35) described the same type of color arrangement in some dermatological isolates and no difference in efficiency between PLM and Sabouraud’s plus cycloheximide and chloramphenicol. Sinkski’s results (39) diverged with 7 of 23 isolates from dermatology patients. False positives and one false-negative reaction were produced on PLM. Using 178 strains of *Candida* from many sources, mostly human, Ridley (33) reported complete correlation between color reactions of PLM and identification by production of chlamydospores, “spidery” colonies, and germ tubes. Gillespie et al. (13), using material from breast and vagina, decided that PLM offered a reasonably accurate method. One culture of *C. albicans* isolated by them on Sabouraud’s medium was not positively identified on PLM out of 142 of yeast isolates. In comparing PLM with Nickerson’s medium, Mendel et al. (23) noted no difference when color and texture of yeast colonies and identification were compared, and also no differences in bacterial contamination on either media. Similar results were reported by Varga (49) in comparing Nickerson’s medium and PLM. The color characteristics of 43 strains of *C. albicans* growing on PLM at 37 C at the end of 24 h were similar to those after 55 h of incubation at room temperature by Kelly et al. (15). Using *Candida (Monilia)* organisms
from the mouth, Cuttita et al. (9) found no instance of a false positive on PLM when “other well known laboratory procedures” were used to verify the identity of the organism. Only one of 33 isolates gave a false-negative reaction on PLM. PLM was more reliable than brain heart medium plus chloramphenicol and cycloheximide for isolating C. albicans from the oral cavity (36). Using PLM for feces and water, Stedham et al. (43) increased the antimicrobial content and noted a somewhat altered color upon growth.

Unlike most previous workers who had found PLM reasonably good for isolating and identifying C. albicans, inhibition of the growth of C. albicans by PLM was reported by Allison (2). Human saliva was the source of the organisms.

Brown-Thomson (7) described 15 morphological forms of C. albicans isolated from a wide variety of sources. Of these, 92% were smooth, shiny, and dome-shaped colonies and were designated “A-1.” Such isolates reduced 2,3,5-triphenyltetrazolium chloride to red or deep red. He cites other workers as having observed the same reaction. Nickerson (25) reported a mutant strain of C. albicans that developed red colonies in a 2,3,5-triphenyltetrazolium chloride-containing medium.

The purposes of this investigation were to determine: (i) whether or not pigment production and colony texture on PLM aided in identification of C. albicans; (ii) if other organisms found in the human vagina produce colors and colonies confusable with C. albicans or other Candida spp.; (iii) whether or not PLM inhibits the growth of C. albicans; and (iv) whether PLM isolates the same Candida spp. as those isolated on Littman’s ox-gall agar and Sabouraud’s medium plus antimicrobials.

**MATERIALS AND METHODS**

**Patient groups and specimen acquisition.** Approximately 500 random clinical samples were taken from the vagina with sterile, saline-moistened cotton swabs, including pregnant and nonpregnant patients as well as patients using and not using oral contraceptives. Immediately after the sample was taken, it was swabbed onto the three test media in random sequence.

**Media used.** “Candida test medium,” supplied by Squibb, was supplied already poured in petri dishes and was refrigerated upon receipt. Sabouraud’s glucose agar with cycloheximide and chloramphenicol was prepared according to Emmons et al. (10). After autoclaving, the medium was cooled to 50 C before adding the antimicrobials. These were cycloheximide (Actidione, Upjohn) in acetone and chloramphenicol in 95% alcohol. Littman’s ox-gall agar was prepared according to the manufacturers directions (Difco); no antimicrobials were added. Sabouraud’s and Littman’s ox-gall agar were refrigerated as soon as the media in petri dishes solidified. All three media were refrigerated until just before the vaginal sample was swabbed onto them. Inoculated media were incubated at room temperature and held together with a large rubber band to prevent drying.

Plates were observed from 3 to 4 days afterwards. Counts were made on each medium noting color and texture of the yeast isolates. In a few instances (~10) one of the three plates became contaminated with fast-growing fungi. In these cases the yeast isolate was transferred to a tube of plain Sabouraud’s medium. The colony counts from this series of three media were not used when colony counts were compared.

**Identification of yeast isolates.** The principal method to identify the yeasts was the dip-slide method with corn meal agar (Difco) with 1% Tween 80 (polysorbate 80) (BBL). If the isolate produced mycelium or pseudomycelium with abundant chlamydospores without development of ascospores, it was called C. albicans. If the organism was nonascosporic and produced mycelium or pseudomycelium without abundant chlamydospores confirmatory fermentation and assimilation reactions were carried out. These tests were performed within 30 days of the original isolation (32).

**Data analysis.** By color and texture the isolates on Candida test medium fell into three groups: (i) white to pink, creamy-textured colonies typical of C. albicans; (ii) red center with a white periphery, creamy textured colonies described for some isolates of C. albicans; (iii) red, salmon pink, deep pink or black, and any colony not creamy in texture, not usually C. albicans. Photographs of typical isolated colonies from each of these three categories are given in Fig. 1.

To determine whether any of the media supported more or less growth of Candida spp. and other yeasts, colony counts were made. Plates whose colonies were too many to count were marked as such, and the actual count was not recorded. After identification those specimens determined to be Candida spp. were categorized as 0, 1-9, and ≥10. To determine bias in establishing these categories for analysis of colony counts, analyses were made of the actual recorded colony counts, omitting those recorded as too many to count. Lot #1 contained those colony counts >10 on each of the media but less than the “too-many-to-count” designation. Lot #2 contained those colony counts 0 to 9 on all three media. This lot was established because of the necessity to transform these low numbers. Lot #3 contained those specimens that produced colony counts not including the too-many-to-count designation, that were not all 10 or greater or 0 to 9 but were a mixture of these two. Because some of these counts were zero, the data obtained were also transformed for analysis. Colony counts of yeast isolates that were not Candida spp. were also tabulated but were not used for the multi-way analysis or the aforementioned analysis.

Multi-way classification of colony-score frequencies with the three media tested was analyzed for independence by using the G-test (41). We also em-
employed this test to test distribution of frequencies of positive specimens classified as to color and texture of colonies on Candida test medium.

To determine whether any one of the three media selectively supported the growth of yeasts that the others might not, notice was taken of colonies on a single plate that had different morphology, e.g., if a plate of Candida test medium had two or three distinctly different colored colonies, identification was made of each such colony. Colonies that differed morphologically on the other media were also subject to individual identification procedures.

RESULTS

Table 1 gives the numbers of yeast isolates from random vaginal samples as categorized in the three color responses on Candida test medium, then identified morphologically or physiologically as C. albicans or not. Because the directions supplied with the Candida test medium state C. albicans colonies can be solid white to pink and also have centers of deep red or purple with a creamy texture, these two color descriptions were placed together for the following calculations. If comparisons are made using the total number of specimens processed (501), the following are the number of specimens and percentages for the various categories: 124 (24.8%) correct positives, 11 (2.2%) false positive, 6 (1.2%) false negatives, and 360 (71.9%) correct negatives. If the comparisons are based on those that yielded C. albicans, a total of 130 samples, 124 (95.4%) were correctly identified on PLM, and 6 (4.6%) produced false-negative reactions. Of the 371 samples not yielding C. albicans, 343 (92.5%) produced no growth; 11 (2.9%) false positives; and 17 (4.6%) true negatives. If additional comparisons are based on the 158 samples that yielded yeasts, number of

**FIG. 1. Color intensities produced by yeast isolates on Candida test medium.**

**TABLE 1. Number of yeast isolates from random vulvovaginal smears on Candida test medium categorized by colony color and texture, then compared with morphological or physiological identification of Candida species**

<table>
<thead>
<tr>
<th>Colony color and texture</th>
<th>No. of isolates</th>
<th>Identification as C. albicans*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pink-white, creamy</td>
<td>119</td>
<td>+; True positive</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>-; False positive</td>
</tr>
<tr>
<td>Red center white peri-</td>
<td>5</td>
<td>+; True positive</td>
</tr>
<tr>
<td>phery, creamy</td>
<td>2</td>
<td>-; False positive</td>
</tr>
<tr>
<td>Red, Salmon pink, black,</td>
<td>6</td>
<td>+; False negative</td>
</tr>
<tr>
<td>pink, not creamy</td>
<td>17</td>
<td>-; True negative</td>
</tr>
<tr>
<td>No growth</td>
<td>343</td>
<td>-; Complete negatives</td>
</tr>
</tbody>
</table>

*On Candida test medium.

*Identification by slide culture with pseudomycelium and chlamydospores in abundance or sugar fermentation and assimilation reactions.
specimens and percentages for the various categories are as follows: 124 (78.5%) correct positives, 11 (7%) false positives, 6 (3.8%) false negatives, and 17 (10.8%) correct negatives.

*Candida* species isolated from samples plated on Sabouraud's, Littman's, and *Candida* test media were grouped as to colony counts and presented in Table 2 as a multi-way classification frequency analysis (41). The colony counts were placed in three groups: ≥10, 1–9, and 0 colonies per plate. For development of Table 2, patient sample numbers were placed in the appropriate colony count column with reference to the results on Sabouraud's medium. Then the results on Littman's medium were compared with the colony counts on Sabouraud's medium. For each patient sample put in a particular colony count group, three comparatives were established for Littman's medium. One of the colony-count groupings resembled that on Sabouraud's medium, but the remaining two were different. A patient number was then placed into one of three possible classes of colony counts as before but in relation to the colony count on Sabouraud's medium. As a result, a total of nine colony-count groups were established for Littman's medium, and the patient sample numbers were classified accordingly in these groups.

The same comparison of colony-count groups was then made for each of those nine colony-count classifications established in the Littman's row. Thus there was a total of 27 colony-count groups for the results on *Candida* test medium. For each patient sample in the colony-count groupings for the Littman's row the colony count that was observed determined into which one of the three colony-count groups the patient sample identification number was placed. After all the patient sample identification numbers had been properly placed, the final counts were used in the *Candida* test row (Table 2).

The ability of Sabouraud's, Littman's, and *Candida* test medium to support growth of yeasts as determined by a comparison of colony counts by using this multi-way technique proved similar. The number of isolations placed into the same colony-count groups on the three media were statistically significant. Thus, all three media supported colony counts of 10 or more, 1 to 9, and 0 similarly. In the remaining 24 colony-count groupings that were dissimilar when compared to each other, the number of isolations were randomly assorted and, therefore, not significant by the G-test.

In the three separate analyses of colony counts to determine whether bias was introduced into the colony-count classification, 40 samples were analyzed. By analysis of variance no significant differences were found among the media. Thus, the categories of colony counts as established for Table 2 were considered reliable.

Five of the samples yielded *C. parapsilosis*. Four of the five strains isolated produced no growth on the Sabouraud's medium plus antimicrobials but growth on Littman's and PLM. One strain produced one colony on Sabouraud's medium with many colonies on the other media. Other yeasts isolated were *Rhodotorula*, pigmented black yeasts, and ascosporic yeasts. Most of these did not grow on Sabouraud's but grew on the other two media. *C. stellatoidea* was not isolated.

In correlating which species of *Candida* grew and was then subsequently identified on the media, there was complete agreement, i.e., the same *Candida* spp. were isolated on the media from a single sample. In some instances, as already mentioned, one of the media did not support growth of some samples. Why in a very few instances *C. albicans* on the same PLM

<table>
<thead>
<tr>
<th>Media</th>
<th>Colony count</th>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sabouraud's dextrose</td>
<td>10 or more (121)*</td>
</tr>
<tr>
<td>Littman's ox gal*</td>
<td>10* (112)</td>
</tr>
<tr>
<td><em>Candida</em> test</td>
<td>10* (108)*</td>
</tr>
</tbody>
</table>

* Containing cycloheximide and chloramphenicol.
* Numbers in parentheses represent the number of cases.
* Without antimicrobials.
* 10 or more.
* Significant at the 95% confidence level.
plate produced solid pink as well as dark red center with white periphery colonies remains unknown.

**DISCUSSION**

If all the random vaginal samples were considered, 1.2% yielded colonies producing a false negative or distinctly red color on PLM and were truly *C. albicans*. When only those samples that produced yeast growth were considered, 3.8% were false negatives. When the same scheme to consider all samples or just those that produced yeasts was used, 2.2 or 7% were false positives, respectively.

PLM supported the growth of *C. albicans* in a manner equal to that of Littman's ox gall agar without antibiotics and Sabouraud's plus antimicrobials, judged from colony counts—a result different from that of Allison (2). We were unable to analyze his data because plate counts and specific information were absent. There did appear to be a consistent, presumably significant, difference in colony counts on PLM when compared to Sabouraud's. The differences might be attributable to the particular commercial source as well as to the fact that the source of cultures was oral saliva. Colony counts can be affected by steps in preparation of agar media. Also, organisms from saliva might differ slightly upon primary laboratory culture than those from the vagina.

Whether PLM favors the growth of certain taxa of yeasts as compared with what may be selected on other media is unreported. When yeast growth was obtained on all three media inoculated from a single vaginal sample, the same taxa of *Candida* were isolated. Although yeasts other than *Candida* species and *Rhodotorula* were not completely identified, morphology appeared similar for isolates from each of the three media. Sabouraud's medium plus antimicrobials was the most inhibitory medium for yeasts other than *C. albicans*.

**LITERATURE CITED**


