Comparative Isolation of Vaginal Yeasts on Selective and Nonselective Media

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The isolation of vaginal yeasts was compared on a selective medium, phosphomolybdic acid agar, and on starch agar, a nonselective differential medium used primarily to isolate *Corynebacterium vaginale*. The majority of the *Candida albicans* strains were isolated on starch agar, but the selective medium was required for isolating all yeasts from the greatest number specimens.

The laboratory diagnosis of vaginal candidiasis is relatively simple when yeastlike fungi are observed in direct wet-mount preparations. However, cultural methods are generally more sensitive than direct microscopy in detecting organisms in any specimen, and culture is considered essential for detecting vaginal yeast infections (14). Several media are available for yeast isolation (11) and for the isolation of *Corynebacterium vaginale* (6). Although there is some uncertainty regarding the pathogenic role of *C. vaginale*, the majority of clinical studies on this bacterium support a causative role in vaginitis (6).

Starch agar (10) is one nonselective differential medium used for isolation of *C. vaginale*, but a variety of vaginal organisms including yeasts grow on the medium. In the venereal disease clinics of Contra Costa County, there has been interest in isolating the yeasts *Trichomonas vaginalis* and *C. vaginale*, in addition to the isolating *Neisseria gonorrhoeae*.

Studies have showed that when fungi are sought in specimens that also contain a mixed bacterial flora, fungi are recovered from more specimens on media selective for fungi (4, 12). This study was conducted to determine the efficacy of starch agar alone as a medium for isolating vaginal fungi in addition to *C. vaginale* in a venereal disease clinic population.

One hundred and seventy-two females were cultured. All patients, who were examined with the aid of a speculum, had some form of vaginal discharge; 38 patients complained of itching or burning, eight had abdominal pain, and one had moderate vaginal bleeding. Ten percent KOH wet-mount preparations were used to detect fungi. A Gram-stained smear was used primarily to aid in detection of clue cells (5), which are squamous epithelial cells stippled with masses of gram-negative pleomorphic coccobacilli. Discharge material was

collected with swabs, rolled down the center of isolation plates, and further cross-streaked with a loop. Starch agar was prepared with purple broth base (Difco) by Smith's method (11) (final pH, 7.2). Phosphomolybdic acid (PMA) agar, selective for fungi, was made according to the method of Bump and Kunz (2) with a final pH of 5.3. Both media were incubated at 37 C in 5% carbon dioxide and observed daily for 3 days. The degree of extent of yeast growth on plate media was scored as follows: 4+, colonies covering 100% of plate; 3+, covering 75% of plate; 2+, covering 50% of plate; 1+, covering 25% of plate; and less than 1+. Yeasts were identified by conventional morphological and biochemical procedures approved and recommended by the California State Health Department. This included detection of chlamydospores, pseudomycelium, urease activity, and carbohydrate fermentations and assimilations. *C. vaginale* was identified by the methods of Dunkelberg et al. (7).

Of the 172 females cultured, 60 (35%) had *C. vaginale*, detected in discharge material by a combination of clue cell detection in smears and cultural methods. Twenty-eight of the 60 patients produced both clue cells and a positive culture, 24 were clue cell negative but culture positive, and 8 were clue cell positive but culture negative. There were 51 patients with mixed infection, which included syphilis, gonorrhea, trichomoniasis, scabies, herpesvirus, venereal warts, and candidiasis. On starch agar, *C. vaginale* was usually the predominant organism, but it frequently occurred with a mixed bacterial flora. There was no bacterial contamination observed on PMA agar from any of the 172 specimens.

There were only two patients from whom both *C. vaginale* and yeasts were isolated. Yeasts were detected in 44 patients. This included 25 positive by wet mounts and culture,

15 positive by culture only, and 4 with positive wet mounts but negative culture. A total of 39 strains of yeasts were isolated on PMA agar, and 31 strains were isolated on starch agar (Table 1). Two strains of *Candida tropicalis*, one strain of *Torulopsis glabrata*, and five strains of *Candida albicans* isolated on PMA agar were not isolated on starch agar. Except for these cases, the growth of yeasts when present on both starch and PMA, agars was approximately equal. Further, the degree or extent of yeast growth on either medium varied, and in general was not related to whether yeasts were seen in wet mounts (Table 2). Six patients had 4+ cultures with negative wet mounts. Conversely, four had positive wet mounts but negative cultures.

C. albicans and possibly other yeasts may be isolated from the genital tract of asymptomatic patients (8). In pregnant patients, however, positive yeasts cultures almost invariably indicate infection (3), and there is not necessarily any relationship between the amount of growth obtained in vitro and the severity of vaginal

TABLE 1. Comparative isolation of yeasts from genital specimens on selective and nonselective media

Yeasts isolated ^a	No. isolated ^b	No. of strains isolated on:	
		PMA agar ^c	Starch agar
<i>Candida albicans</i>	35	34 (97%)	29 (82%)
<i>Candida tropicalis</i>	2	2	0
<i>Torulopsis glabrata</i>	4	3	2
Total	41	39 (95%)	31 (75%)

^a Isolated from 40 patients.

^b Based on isolation of organisms from both media.

^c Selective for fungi.

TABLE 2. Relationship between yeasts observed by direct microscopic examination and extent of in vitro growth^a

Extent of growth	Group A ^b	Group B ^c
4+	11	6
3+	6	1
2+	6	3
1+	2	2
<1	1	2

^a Yeasts isolated from group A included 25 *Candida albicans*, one *C. tropicalis*, and one *Torulopsis glabrata*. Group B isolates consisted of 10 *C. albicans*, one *C. tropicalis*, and three *T. glabrata*. Four patients had positive wet mounts but negative culture.

^b Twenty-six patients, wet mount and culture positive.

^c Fourteen patients, wet mount negative and culture positive.

infection (9). Similarly, as in this study, there was no consistency between the detection of yeasts in discharge material by wet mounts and the extent of in vitro growth.

Although Thayer-Martin (TM) agar (widely used for selective isolation of *Neisseria*) contains 12.5 U of nystatin per ml to inhibit yeasts, at least some isolates of *C. albicans* are known to grow on this medium (10); therefore, clinicians or microbiologists who seek information on the presence of yeasts in vaginal specimens may use TM agar both for yeasts and gonococcus isolation. The extent to which this practice is used and is microbiologically sound is not known. There is wide variation in the susceptibility of *C. albicans* and other yeasts to nystatin (1, 13) and little information to predict the rate of yeast isolation on TM agar as opposed to a more suitable yeast isolation medium.

Yeasts other than *C. albicans* are known to cause or contribute to vaginitis (3, 8, 9). Culture is obviously necessary to detect yeasts in the greatest number of specimens, and in our clinics this information almost invariably leads to treatment. PMA agar was an inexpensive and simple medium to prepare for yeast isolation, and it recovered yeasts from 23% of the 172 patients. Yeasts were isolated from 18% of the patients on starch agar. In clinics where the choice of media for culturing genital tract pathogens may be limited, we believe that starch agar is preferable because of its utility in isolating most yeasts and providing rapid differentiation of *C. vaginale* from other vaginal organisms. The efficacy of still other combinations of media to culture vaginal pathogens is being studied.

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