Evaluation of a dehydrated test strip for the detection of yeasts

R. R. DAVIES AND M. A. SAVAGE

From the Department of Bacteriology, St Mary's Hospital Medical School, Norfolk Place, London W2 1PG

SYNOPSIS Use of a dehydrated test strip for the detection of yeasts is compared with traditional culture on Sabouraud's agar containing 50 µg/ml chloramphenicol. While the selective medium of the strip is satisfactory for the isolation of species of *Candida*, *Torulopsis glabrata* grows only very slowly. The strip has the advantage of a long storage life without deterioration, but a high cost may preclude general usage. The numbers of yeasts collected by a bacteriological swab, disadvantages of the selective medium, and the value of direct microscopy in the examination of vaginal swabs are discussed.

Among a variety of tests for the detection of bacteriuria without recourse to the laboratory are the dip spoon of Mackey and Sandys (1965) and the dip slide of Naylor and Guttman (1967). In these, a plastic holder which supports an agar medium is simply dipped into the urine and then replaced in a container for incubation or dispatch through the mail for examination elsewhere. The Microstix (Ames Co.) represents a later development in which the agar medium is dehydrated so that storage of the test strips is simplified and shelf life lengthened. The study to be described evaluates a similarly supported, dehydrated agar medium for the detection of *Candida albicans*, Microstix Candida (MC). In this, since the dehydrated selective medium may be inoculated with a bacteriological swab, its use may differ from the tests for bacteriuria. The kit comprises radiation-sterilized MC sticks, each in a transparent envelope, a dropping bottle of rehydration fluid, and self-sealing plastic pouches in which the sticks are incubated. Before inoculation, the 10.5 mm square medium impregnated pad is first moistened with 2 drops of the rehydration fluid. Inoculation is by rolling the swab over the surface of the pad, and immediately afterwards the strip is inserted in a plastic envelope, which is sealed for incubation. The patient's name or details may be written on the pouch with a felt-tipped pen.

Materials and Methods

In the MC, Nickerson (1953) medium suspended in

Received for publication 17 February 1975.

alginate is absorbed in a pad of porous cellulose and bonded to the end of a clear plastic strip. The efficacy of the moistened pad was compared with that of the routinely used Petri dish of Sabouraud's dextrose agar containing 50 µg/ml chloramphenicol.

High vaginal swabs were numbered on reception, and odd numbers were cultured by rubbing over $\frac{1}{8}$ sector of the surface of a Sabouraud's agar plate and then on to the MC. Even numbered swabs were first inoculated on MC, as described above, and then on to the Sabouraud's agar. Cultures were incubated for not less than 24 hours at 37°C and the MC was examined for colour change. In addition to cultural examination, the swabs were also used to prepare wet films for direct microscopy.

From a suspension of *C. albicans* in distilled water decimal dilutions to 10⁻⁷ were prepared, and viable counts were undertaken for each dilution. Into each dilution swabs were dipped and then drained against the side of the tube before culture on MC or Sabouraud's agar. The number of yeasts taken up by a swab was calculated by weighing the volume of water absorbed and dividing this by the number of viable blastospores per millilitre of suspension.

Results

A total of 250 high vaginal swabs was examined, and the results are given in table I.

Direct microscopy detected less yeast carriage than either the MC or Sabouraud's agar. Culture on Sabouraud's agar, which was the most sensitive

Grown on Sabouraud's agar	100
Grown on MC	89
Direct microscopy	78

Table 1 Number of swabs with yeasts

technique, yielded 11 more yeast carriers than MC. However, in these cases 1 to 4 colonies only were isolated. The MC detected two carriers who were not found by culture on Sabouraud's agar.

The mean weight of water absorbed by the swabs employed was 0.13 g (SD 0.03 g).

The calculated number of yeasts absorbed by swabs dipped in dilutions of a suspension containing 6.5×10^5 blastospores, with the respective numbers of colonies isolated by rubbing the swabs on Sabouraud's agar and MC, is given in table II.

Calculated No. of Yeasts absorbed by Swabs	No. of Colonies Developed	
	Sabouraud's Agar	MC
6.5×10^5	++	++
6.5×10^4	+	+
65	5	3
6.5	2	2

Table II Number of yeasts absorbed by swabs and number of colonies which developed after the swabs were rubbed on agar and incubated

++ semi-confluent growth; + 50-100 colonies

Vaginal yeast carriage occurred in 41% of the 250 patients examined; 83 carried *C. albicans*, nine *C. tropicalis*, three *C. parapsilosis*, and two *Torulopsis glabrata*. In five patients carrying *C. albicans*, two also carried *T. glabrata*, two *C. tropicalis*, and one *C. parapsilosis*. When tested separately on the MC, all the *Candida* species grew well, but variable results were obtained with *T. glabrata*.

On the BiGGY medium (DIFCO Laboratories) of Nickerson (1953) the colony of *T. glabrata* grows much more slowly than that of *C. albicans*. After 24 hours on this medium, which contains a bismuth sulphite indicator, the colonies of *T. glabrata* are so small that they are barely discernible with the unaided eye, and 600 colonies per square centimetre of surface area showed no perceptible colour change.

Discussion

Although all the *C. albicans* collected by a bacteriological swab dipped in a suspension are not deposited when the swab is rubbed over a surface, similar

numbers grew on Sabouraud's agar and on the alginate pad of the MC (table II).

Culture on Sabouraud's agar showed 11 more yeast carriers than culture on MC (table I), but in these cases from 1 to 4 colonies only were isolated. Selective media tend to be inhibitory to the growth of the organism they are designed to select, and while no inhibition occurred when *C. albicans* in exponential multiplication was inoculated on Nickerson's medium (1953), a small population of marasmic cells might not develop. The fact that the MC failed to detect *C. albicans* when it grew in small numbers on Sabouraud's agar might be an advantage in the investigation of urine where the presence of small numbers of yeasts is not diagnostic of a clinically significant urinary infection. This is particularly true in the female, where contamination from the vagina can lead to small numbers of *C. albicans* being cultured from the urine. Kass (1956) reports 10^5 *Escherichia coli* as being diagnostic of clinical bacteriuria, and similar counts are observed in candiduria. Since the alginate pad absorbs 0.1 ml (SD 0.001) of water, a 10^4 viable count per millilitre of urine will give 10^3 colonies on the pad and a colour change of the entire surface after 24 hours' incubation. However, use of the MC as a dip technique for urinary infections might give anomalous results with *T. glabrata*. In 63 yeast infections of the urinary tract with 10^5 colonies per ml or more, 53% were due to *C. albicans* and 34% were caused by *T. glabrata* (Davies, unpublished). This organism does not produce a colour change in the pad after 24 hours, and to provide for its detection the MC should be incubated for another 24 hours. A trial of the MC with urine specimens is not practicable because urinary infections with yeasts are rare, only 63 being detected in over 28 000 urine specimens quantitatively cultured (Davies, unpublished).

The assemblage of yeasts isolated from high vaginal swabs in this study was similar to that recorded by Hurley *et al* (1973). The swabs were those received by a diagnostic service, and the yeast isolation rate of 41% lay between that of 8.7% from hospital patients in Edinburgh (McKenzie, 1961) and 84% in pregnant women with clinical signs of vaginitis or vulvovaginitis (Carroll *et al*, 1973).

The practitioner whose sole concern is to determine whether or not a vaginal discharge contained yeasts, regardless of the cost of the test, may find the MC of value. The long storage life facilitates the purchase of a quantity from which only occasionally a single MC may be used. The fact that a variety of yeasts may cause an infection and that the MC fails to discriminate between species of *Candida* is less important than the numbers of yeasts present, since

species specific resistance to antifungal drugs has not yet emerged as a significant factor in therapeutic failure in vaginitis.

On the other hand, a microscope may be of greater value. Although it may be seen (table I) that direct microscopy failed to detect as many carriers of yeasts as did the cultural techniques, in the diagnosis of infection where there is a significant yeast growth, direct examination should invariably give a positive result. Microscopic examination of a wet mount shows blastospores and mycelium in the *Candida* infections, and, when present, the motile protozoan *Trichomonas vaginalis* can be easily recognized. Hurley *et al* (1973) report that, in comparison with two staining methods, microscopic examination of wet smears for *T. vaginalis* gave satisfactory results. With direct examination, evidence of fungal, protozoan or concomitant infections may be obtained while the patient awaits a prescription based on the microscopic findings.

The cost of the MC¹ is likely to prohibit any general usage in Great Britain, where in the National Health Service the general practitioner may send specimens to a laboratory in which the cost of a poured Petri dish of Sabouraud's agar with 50 µg/ml chloramphenicol is approximately 10 pence. In the diagnostic laboratory a number of swabs will be cultured for *Candida* each day and several patients'

specimens may be examined in the same Petri dish of agar. The MC has little to commend it for use in the medical microbiology laboratory.

A single culture medium which facilitates both isolation and the specific identification of yeasts has yet to be described, and mycologists will prefer isolation, subculture, and identification by traditional methods.

We are grateful to the Ames Co for a generous supply of Microstix Candida.

References

- Carroll, C. J., Hurley, R., and Stanley, V. C. (1973). Criteria for diagnosis of candida vulvovaginitis in pregnant women. *J. Obstet. Gynaec. Brit. Cwlth*, **80**, 258-263.
- Hurley, R., Leask, B. G. S., Factor, J. A., and de Fonseka, C. I. (1973). Incidence and distribution of yeast species and of *Trichomonas vaginalis* in the vagina of pregnant women. *J. Obstet. Gynaec. Brit. Cwlth*, **80**, 252-257.
- Kass, E. H. (1956). Asymptomatic infections of the urinary tract. *Trans. Ass. Amer. Physns*, **69**, 56-64.
- McKenzie, D. W. R. (1961). Yeasts from human sources. *Sabouraudia*, **1**, 8-15.
- Mackey, J. P. and Sandys, G. H. (1965). Laboratory diagnosis of infections of the urinary tract in general practice by means of a dip-inoculum transport medium. *Brit. med. J.*, **2**, 1286-1288.
- Naylor, G. R. E. and Guttman, D. (1967). The dip slide. *J. Hyg. (Camb.)*, **65**, 367-371.
- Nickerson, W. J. (1953). Reduction of inorganic substances by yeasts; 1. Extracellular reduction of sulphite by species of *Candida*. *J. infect. Dis.*, **93**, 43-56.

¹10 US dollars per kit of 25 tests