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MYCOLOGY

# An evaluation of the cost-effectiveness of using CHROMagar for yeast identification in a routine microbiology laboratory

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**CHROMagar, a chromogenic differential culture medium, is claimed to facilitate the isolation and presumptive identification of certain clinically important yeast species, e.g., *Candida albicans*. This study evaluated the cost-effectiveness and time advantage of using it in comparison with Sabouraud dextrose agar (SDA). Three possible pathways, each of which included the use of one or both media, were compared in a routine laboratory. A total of 21 yeast isolates was cultured from 298 clinical samples from neutropenic and AIDS patients. An overall sensitivity of 95.2% was observed for each medium and primary isolation on CHROMagar was found to be 100% sensitive and 100% specific for *C. albicans*. For identification purposes, after initial culture the use of CHROMagar provided the most economical and least time-consuming method. Direct inoculation on to CHROMagar is recommended for blood cultures when yeast cells are seen on microscopy and where early appropriate therapy is imperative.**

## Introduction

In routine diagnostic laboratories Sabouraud dextrose agar (SDA) is widely used for the isolation of all yeast species from clinical specimens [1]. SDA is a selective medium supporting the growth of most pathogenic fungi encountered in clinical samples. In some laboratories an indicator dye (bromocresol green) is added to the SDA medium, producing a green solid agar that allows strain differentiation. The SDA agar is usually incubated for 48 h to isolate yeasts from a clinical sample, although *Cryptococcus neoformans* may require several weeks.

*Candida albicans* is the yeast species isolated most often from clinical material [2, 3] and is frequently of clinical importance. However *C. krusei* and *C. glabrata* are now being encountered with increasing frequency, and may be associated with the prophylactic use of fluconazole [4, 5], to which they are less susceptible. Therefore, identification has important therapeutic implications. Mixed cultures containing these organisms might also explain problems with therapy. However, epidemiological studies have shown mixed cultures containing *C. albicans* in $< 10\%$ of samples [6].

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Most clinical laboratories use the formation of a germ-tube in serum [7] as the initial test to differentiate *C. albicans* from other yeast species. This is then followed by more time-consuming and expensive biochemical identification [2] usually in the form of a commercially available kit. To fully identify a yeast may take up to 72 h from primary isolation of the organism.

CHROMagar Candida (CA), a yeast differential and selective medium, allows the recognition of mixed yeast cultures in clinical specimens [3]. The medium permits the presumptive identification of *C. albicans* from other *Candida* spp. Yeast populations are differentiated by colonial morphology and colours which are generated by a chromophore in the agar [3]. *C. albicans* strains produce $\beta$-N-acetylgalactosaminidase, which interacts with the chromophore (chromogenic hexosaminidase substrate) incorporated into the agar, and with incubation for 48 h produces green colonies, characteristic of all *C. albicans* isolates [2]. Other *Candida* spp. can also be differentiated, e.g., *C. tropicalis* produces purple colonies.

The objective of this study was to examine the use of CA as the primary or follow-up medium for the culture and identification of yeasts in a routine laboratory. The cost and time required to determine the full identification of a yeast isolate cultured from routine clinical specimens were studied in three different process pathways.

## Materials and methods

The investigation was done over 4 weeks on throat, mouth, oesophageal and stool samples taken from leukaemic and AIDS patients.

Sabouraud Dextrose Agar (Unipath, Basingstoke), and CHROMagar (CHROMagar, Paris, France) were prepared following the manufacturers' instructions, in petri dishes holding 15-ml volumes of the liquid agar; 5-ml volumes of CA were prepared in the same manner but poured in 5-ml petri dishes. Plates prepared from both media were stored at 4°C until used.

In the routine microbiology laboratory, 298 clinical specimens from both sets of patients were inoculated on to one quarter of an SDA plate and one quarter of a CA plate and incubated aerobically for 24 h at 37°C. If cultures were negative, plates were incubated for a further 24 h at 37°C. Colony counts were performed on all positive isolates. Three possible pathways for identifying the positive yeast isolates were examined and are illustrated in Figs. 1, 2 and 3. The cost of labour for each pathway was determined from the average (UK) salary of a biomedical scientist. The costs of testing germ-tube production, culture of a swab specimen and performing an API (bioMerieux) identification test were calculated with Welcan units [8]. Inoculation of a swab specimen takes 3.00 units, a germ tube 5.00 units and an API test 4.00 units.

## Results

Twenty-one yeast isolates were cultured from the 298 clinical samples. Some of the isolates were cultured from the same patient, as repeat specimens were often examined to detect the clearance of the organism cultured in primary specimens. Mixed fungal cultures were not obtained with either selective medium. Each medium failed to support the growth of one isolate (cultured on the other), giving an overall sensitivity of 95.2% for each medium.

The germ-tube test was found to have a sensitivity and specificity of 84.6% and 100%, respectively. The growth of green colonies on CA was 100% sensitive and 100% specific for *C. albicans*. Table 1 shows the yield and characteristics of each yeast isolate cultured. Fig. 1 illustrates process pathway 1, showing the stages taken in identifying a yeast isolate by traditional methods. Fig. 2 shows the stages in identifying a yeast isolate when the SDA plate was used for primary inoculation and CA for identifying the *C. albicans* isolates. Fig. 3 illustrates the process pathway when CA was used for primary inoculation and for identification of any *C. albicans* isolates.

Table 2 illustrates the cost of labour, materials and minimum time taken to identify *C. albicans* and *Candida* spp. by pathways 1, 2 and 3.

## Discussion

The yield (i.e., the number of colonies) of yeast strains on CA was greater than on SDA, as found in a study by Baumgartner *et al.* [9], which also demonstrated a detection rate of yeast on CA 20% higher than on SDA. However, there was no difference in the sensitivity (i.e., detection rate) of CA and SDA for primary culture, in keeping with other studies [2, 3].

**Table 1.** Yield and characteristics of 21 yeast isolates cultured from clinical specimens inoculated on to Sabouraud dextrose agar (SDA) and CHROMagar (CA)

| Isolate no. | Specimen | CA colour | CA growth | SDA colour | SDA growth | Germ-tube | Identification |
|---|---|---|---|---|---|---|---|
| 1 | Throat swab | Purple | +/− | Cream | + | Negative | *C. glabrata* |
| 2 | Throat swab | No growth | − | Cream | +/− | Negative | *C. inconspicua* |
| 3 | Mouth ulcer | Green | + | Cream | + | Positive | *C. albicans* |
| 4 | Throat swab | Green | +++ | Cream | + | Positive | *C. albicans* |
| 5 | Stool | Purple | +++ | Cream | + | Negative | *C. inconspicua* |
| 6 | Stool | Purple | + | No growth | − | Negative | *C. inconspicua* |
| 7 | Stool | Green | +/− | Cream | + | Positive | *C. albicans* |
| 8 | Throat swab | Green | +++ | Cream | +++ | Negative | *C. albicans* |
| 9 | Stool | Green | + | Cream | + | Positive | *C. albicans* |
| 10 | Stool | Purple | +/− | Cream | +/− | Negative | *C. inconspicua* |
| 11 | Throat swab | Purple | +/− | Cream | + | Negative | *C. inconspicua* |
| 12 | Throat swab | Green | +++ | Cream | + | Positive | *C. albicans* |
| 13 | Throat swab | Green | +/− | Cream | + | Positive | *C. albicans* |
| 14 | Mouth swab | Green | +++ | Cream | + | Positive | *C. albicans* |
| 15 | Oesophageal fluid | Green | +++ | Cream | +++ | Positive | *C. albicans* |
| 16 | Stool | Green | ++ | Cream | + | Positive | *C. albicans* |
| 17 | Stool | Green | + | Cream | + | Positive | *C. albicans* |
| 18 | Stool | Purple | +/− | Cream | +/− | Negative | *C. inconspicua* |
| 19 | Stool | Green | + | Cream | +/− | Positive | *C. albicans* |
| 20 | Throat swab | Pink | + | Cream | +/− | Negative | *C. glabrata* |
| 21 | Stool | Green | + | Cream | +/− | Positive | *C. albicans* |

−, no growth; +/−, 1–10 colonies; +, 11–40 colonies; ++, 41–100 colonies; +++, 101–200 colonies.

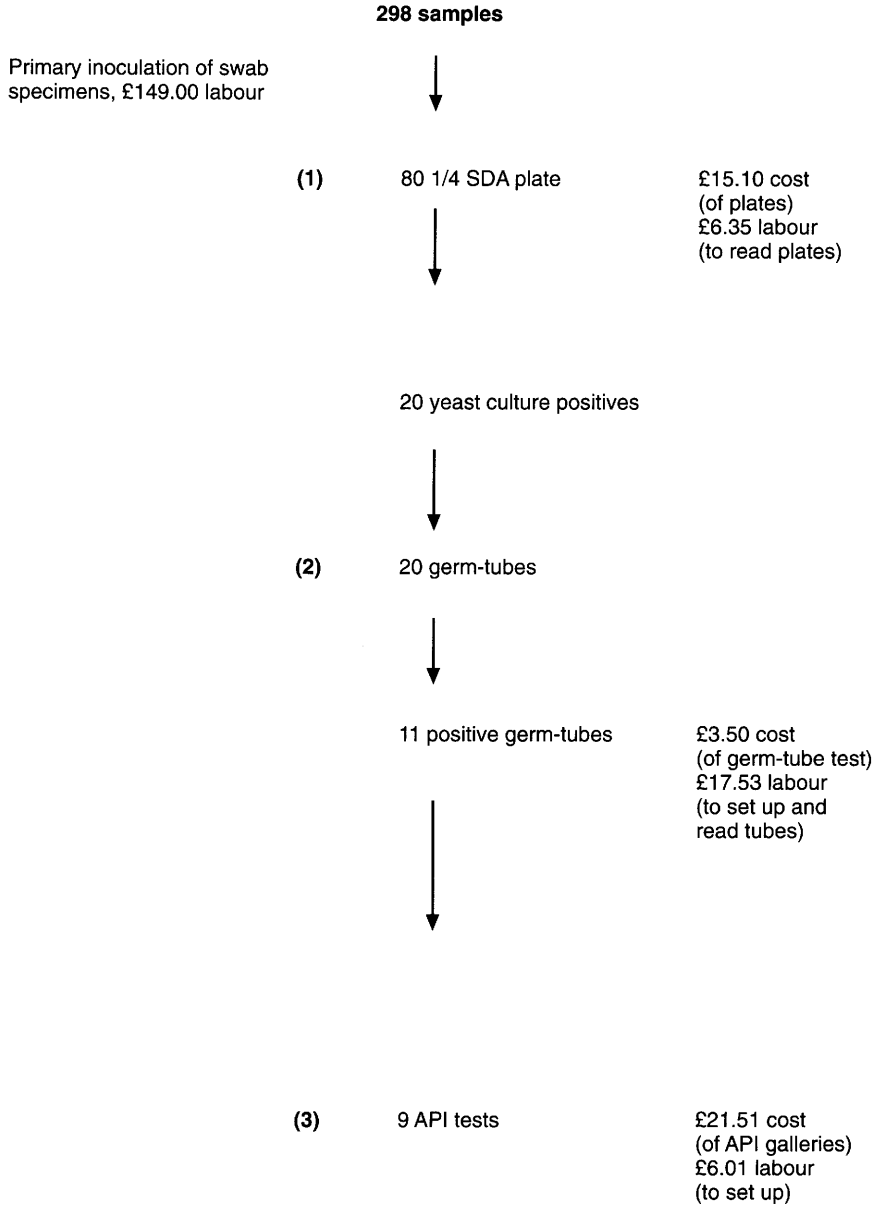


**Fig. 1.** Pathway 1. Steps: **1**, culture of swab on to a 1/4 SDA plate, incubated aerobically for 24 h at 37°C; **2**, germ-tube test performed on all yeast isolates; **3**, API test performed on all germ-tube-negative isolates.

There was a 100% correlation of CA with the API test for identification of *C. albicans* whether determined by primary inoculation or follow-up, which confirms other studies [10, 11].

We are unable to comment on the sensitivity and specificity of the method for the non-*C. albicans* species, as an unusually high percentage of *C. inconspicua* was isolated. Other studies show good specificity for *C. glabrata, C. tropicalis* and *C. krusei*. San-Millan *et al.* [11] showed 100% specificity and sensitivity for *C. krusei* and 93.8% and 99.1%, respectively, for *C. tropicalis*. Bernal *et al.* [10] also showed 99% and 100% specificity for *C. tropicalis* and *C. krusei*, respectively. Odds and colleagues [3] found sensitivities exceeding 99% for both *C. krusei* and *C. tropicalis*.

Two isolates produced negative germ-tube results but were found to be *C. albicans* by biochemical identification. Given the observer variability encountered with the germ-tube test, as well as its lower sensitivity and specificity, CA provides a simple and precise means for rapid identification of *C. albicans* in the routine laboratory.

The time taken to identify *C. albicans* following pathway 1 was *c.* 30 h. This is solely dependent on whether the germ-tube test is performed at 24 h after sample inoculation. The time scale of 30 h may vary

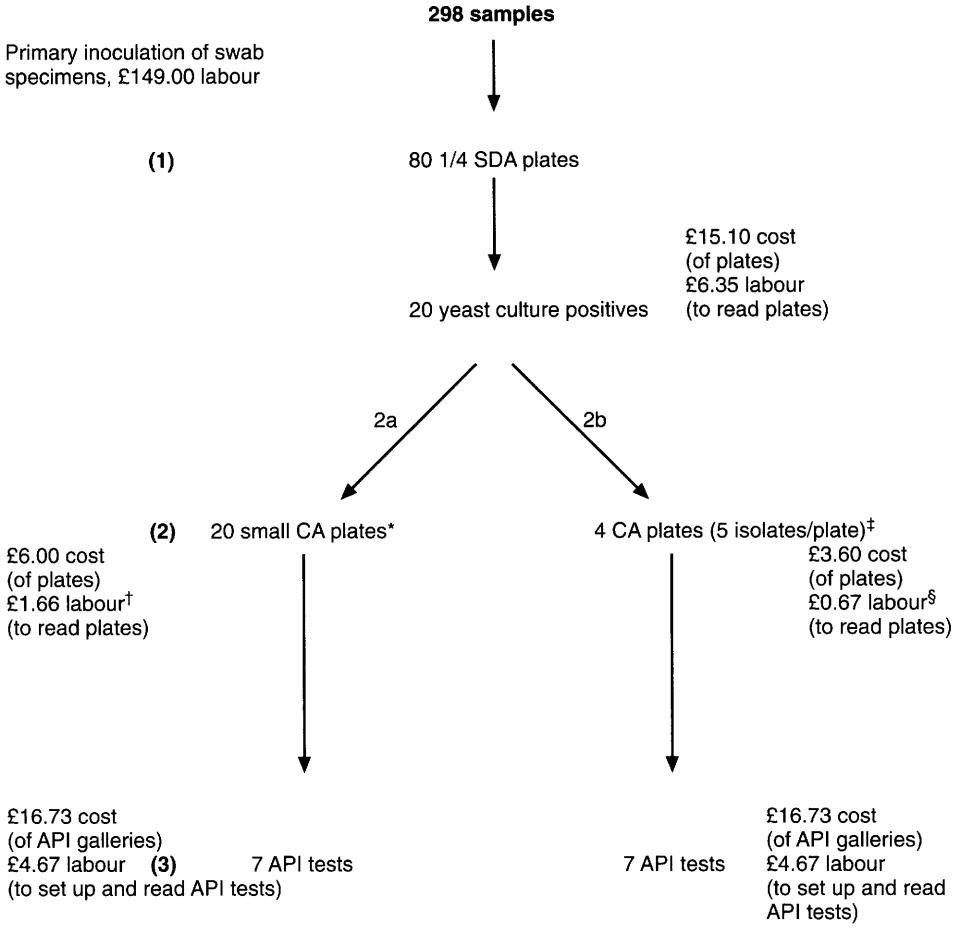


**Fig. 2.** Pathway 2. Steps: **1**, culture swab on to a 1/4 SDA plate, incubated for 24 h at 37°C; **2**, culture of yeast isolates on to a CA plate; **3**, API test performed on all non-green colony types. *CA agar set up at 10.00 am and read at 4.00 pm. †Based on reading 20 individual plates, *c.* 30 s to read a plate; ‡Yeast isolates collected and tested on the CA plate twice a week; §based upon the cost of reading four plates inoculated with the 20 isolates.

according to the time taken for an individual to 'read' a routine batch of specimens, and also if a mixed culture of yeasts is obtained, a pure culture would have to be obtained before any diagnostic test is carried out.

Following pathway 2a, *C. albicans* could be identified within 30 h after sample inoculation. The time here is also dependent on whether a pure isolate is cultured initially and whether the yeast isolate is inoculated on to a CA plate 24 h after primary culture. All isolates producing green colouration within 6 h were confirmed as *C. albicans* by biochemical identification. Pathway 2b was calculated to be insignificantly less expensive than 2a; however, the time taken to identify the yeast strains isolated following this method could take up to 72 h longer, because the isolates would have been collected and identified in two batches per week.

Pathway 3 took the shortest time to identify *C. albicans*.

The most economical method is to use CA for identification only (pathway 2). In laboratories with large numbers of isolates, daily inoculation of plates allows *C. albicans* identification within 30 h, commensurate with current methodology employing germ-tube production. Additional costs of processing these samples by pathway 3 in our laboratory, compared with conventional methods would be UK £385.71 per year. The advantages of using this method in some laboratories may outweigh this modest additional cost. Certainly, primary inoculation of blood cultures on to CA (pathway 3) when yeasts are seen on microscopy would gain time where early appropriate therapy is critical.

In conclusion, CHROMagar provides a simple and inexpensive method for the identification of yeasts. This should allow laboratories to introduce identification of mucosal isolates, now increasingly required and recommended [12], with minimal impact on workflows and laboratory budgets.

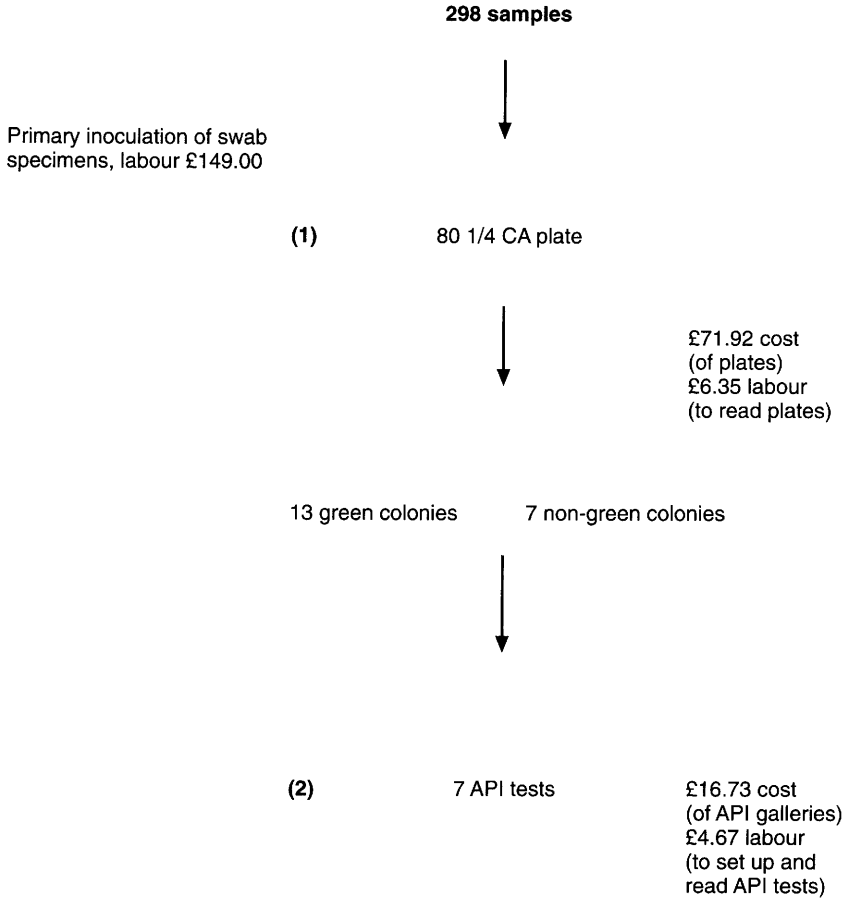


**Fig. 3.** Pathway 3. Steps: **1**, culture of swab on to a 1/4 CA plate for 24 h at 37°C aerobically; **2**, API test performed on all non-green colony types.

**Table 2.** Characteristics of pathways 1, 2 and 3

| Pathway | Labour cost (£) | Cost of materials (£) | Total cost (£) | Minimum time taken to identify *C. albicans* (h) | Minimum time taken to identify *Candida* spp.* (h) |
|---|---|---|---|---|---|
| 1 | 178.89 | 40.11 | 219.00 | 30 | 72 |
| 2a/2b | 161.68/160.69 | 37.83/35.43 | 199.51/196.12 | 30†/30‡ | 72/72§ |
| 3 | 160.02 | 88.65 | 248.67 | 24 | 72 |

*Assuming 42–48 h API incubation, i.e., strips read and identification successful after two periods of overnight incubation.
†Inoculating plates at 10.00 am allows green colouration to be detected at 4.00 pm.
‡Some batched isolates will take 102 h.
§Some batched isolates will take 144 h.

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## References

1. Odds FC. Sabouraud('s) agar. *J Med Vet Mycol* 1991; **29**: 355–359.
2. Beighton D, Ludford R, Clark DT *et al.* Use of CHROMagar Candida medium for isolation of yeasts from dental samples. *J Clin Microbiol* 1995; **33**: 3025–3027.
3. Odds FC, Bernaerts R. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *J Clin Microbiol* 1994; **32**: 1923–1929.
4. Louwagie B, Surmont I, Verhaegen J, Odds F. Differential and enrichment media for selective culture and recognition of yeast species from clinical material. *Eur J Clin Microbiol Infect Dis* 1995; **14**: 406–411.
5. Pfaller MA, Houston A, Coffmann S. Application of CHROMagar Candida for rapid screening of clinical specimens for *Candida albicans, Candida tropicalis, Candida krusei and Candida glabrata* (*Torulopsis*). *J Clin Microbiol* 1996; **34**: 58–61.
6. Odds FC. Candida and candidosis, 2nd edn. London, Bailliere-Tindall. 1988: 68–92.
7. Mackenzie DWR. Serum germ tube production of *Candida albicans. J Clin Pathol* 1962; **15**: 563–565.
8. Welcan UK. Workload measurement system manual, 1992 edn.
9. Baumgartner C, Freydiere A-M, Gille Y. Direct identification and recognition of yeast species from clinical material by using albicans ID and CHROMagar Candida plates. *J Clin Microbiol* 1996; **34**: 454–456.
10. Bernal S, Martin Mazuedos E, Garcia M, Aller Al, Martinez MA, Gutierrez MJ. Evaluation of CHROMagar Candida medium for the isolation and presumptive identification of species of *Candida* of clinical importance. *Diagn Microbiol*

*Infect Dis* 1996; **24**: 201–204.

11. San-Millan R, Ribacoba L, Ponton J, Quindos G. Evaluation of a commercial medium for identification of *Candida* species. *Eur J Clin Microbiol Infect Dis* 1996; **15**: 153–158.
12. Working Parties of the British Society for Antimicrobial Chemotherapy and the British Society for Medical Mycology 1996. Fungal infections: a survey of laboratory services for diagnosis and treatment. *Commun Dis Rep CDR Review* 1996; **6**: Review no. 5.