Cost–effectiveness and efficacy of CHROMagar™ Candida medium in clinical specimens

O.M. Momani [1]

مردودية ونجاعة مستنبت الكروم – أغار للمبيضات في فحص العينات السريرية

عمر موسى المومني

خلاصة: مستنبت الكروم – أغار للمبيضات هو مستنبت جديد يُستعمل في الاستفراد (العزل) التفريقي واستعراف بعض أنواع المبيضات المهمة سريرياً. وفي هذه الدراسة تقييم لمردودية هذا المستنبت مقارنةً بالطُرُق التقليدية. فقد تم بحث ثلاثين ذرية مرجعية و158 عينة سريرية و105 مزرعة مجمعة. وقد زُرعت العينات على مستنبت الكروم – أغار للمبيضات وعلى أغار سابورو بالكلورامفينيكول. وتم التعرُّف بالطُرُق التقليدية على أغار سابورو، وظهور المستعمرات على وسط الكروم – أغار للمبيضات. وقد كشف مستنبت الكروم – أغار للمبيضات كشفاً صحيحاً عن معزولات من المبيضات البيضاء والمبيضات المدارية والمبيضات الكروزية. كما تميَّز هذا المستنبت في التعرُّف على المزارع المختلطة. وقد أُجريت مقارنة من حيث الوقت والتكاليف، وتبين أن مستنبت الكروم – أغار للمبيضات يهيئ طريقة بسيطة ودقيقة وذات مردود جيد مقابل تكلفتها، من أجل التعرُّف على بعض أنواع المبيضات المهمة سريريا.

ABSTRACT CHROMagar™ Candida is a new medium for the differential isolation and identification of certain clinically important *Candida* species. This study evaluated the cost–effectiveness of this medium compared with conventional methods. Thirty reference strains, 158 clinical specimens and 105 stock cultures were investigated. Specimens were cultured on CHROMagar™ Candida medium and on Sabouraud chloramphenicol agar. Identification was by conventional methods on Sabouraud agar and appearance of colonies on CHROMagar™ Candida medium. CHROMagar™ Candida correctly identified isolates of *C. albicans*, *C. tropicalis* and *C. krusei*. It was superior in detecting mixed cultures. A comparison of time and cost was carried out. CHROMagar™ Candida provides a simple, accurate and cost-effective method for identifying some clinically important *Candida* species.

Rentabilité et efficacité du milieu CHROMagar Candida dans les échantillons cliniques
RESUME Le CHROMagar Candida est un nouveau milieu pour l'isolement différentiel et l'identification de certaines espèces de *Candida* importantes sur le plan clinique. Cette étude a évalué la rentabilité de ce milieu par rapport aux méthodes conventionnelles. Trente souches de référence, 158 échantillons cliniques et 105 cultures souches ont été examinés. Les échantillons ont été mis en culture sur le milieu CHROMagar Candida et sur le milieu de Sabouraud-chloramphénicol. L'identification a été effectuée par les méthodes conventionnelles sur milieu de Sabouraud et l'apparition des colonies sur milieu CHROMagar Candida. Le CHROMagar Candida a identifié correctement des isolats de *C. albicans*, *C. tropicalis* et de *C. krusei*. Il était supérieur dans la détection des cultures mixtes. Une comparaison du temps et du coût a été réalisée. Le CHROMagar Candida fournit une méthode simple, précise et rentable pour l'identification de certaines espèces de *Candida* importantes sur le plan clinique.

[1]*Department of Microbiology, King Hussein Medical Centre, Amman, Jordan.*
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Introduction

Candida spp. infections are a growing medical problem as a result of modern medical practice and increasing numbers of immunocompromised patients. Several species previously considered nonpathogenic have emerged as new pathogens. Many types have acquired a high degree of resistance to antifungal therapy [*1–6*].

Rapid, reliable and cost-effective methods of detecting and identifying yeasts allow appropriate treatment to be implemented, which thus reduces morbidity and mortality in those at risk. Yeasts cultured on Sabouraud agar often appear very similar and it is not easy to distinguish between different species if they are present in mixed cultures [*8–10*]. Morphological methods, such as the germ tube (GT) test, are considered the simplest and most valuable means of rapid presumptive identification of *Candida albicans* to distinguish it from other *Candida* species. The definitive identification of *Candida* to the species level is achieved by the carbohydrate assimilation test, which measures the ability of the organism to use specific carbohydrates as the sole source of carbon in the presence of oxygen; results can be obtained 48–72 hours after primary isolation [*11*].

CHROMagar™ Candida (CA) medium (CHROMagar Microbiology, Paris, France) was first described by Odds and Bernaerts in 1994 [*9*]. It is a commercial culture medium containing species-specific chromogenic substrates that allow individual colonies to be identified by colour development and colony morphology during growth. When incubated at 37 °C for 48 hours on CA medium, colonies of *C. albicans* appear green, *C. tropicalis* has distinctive dark blue colonies surrounded by a purple halo in the agar, and *C. krusei* has rough, spreading pale pink colonies with white edges. Other *Candida* species have a variety of colony colours ranging from white to deep pink and purple. All species grow well on CA medium after incubation at 37 °C for 24 hours; however, growth and colour development is most consistent and comparable after 48 hours of incubation [*9*]. Pfaller and colleagues accurately identified 100% of their stock cultures of *C. albicans*, *C. tropicalis* and *C. krusei*. In addition, they identified 90% of *C. glabrata*, which appeared as dark pink colonies with pale edges [*12*]. Using colony colour contrast, the medium facilitates the rapid discrimination of different species in mixed cultures [*9,10,12,13*].

This study evaluated the efficacy and cost–effectiveness of using CA medium as a rapid differential isolation medium for presumptive identification of clinically important *Candida* species from clinical specimens.

Materials and methods

The work was performed in the microbiology laboratories at the University Hospital, Cardiff, Wales, United Kingdom (UK).

A total of 294 specimens and stock cultures was used in the study, including 30 reference yeast strains (Table 1), 105 stock *C. albicans* strains and 158 clinical specimens consisting of 91 urine samples, 66 genital samples (high vaginal, penile and vulvovaginal swabs) and 1 blood culture sample.

The CA medium was purchased from M-Tech Diagnostic Limited, UK and another version of the medium (MAST ID-CHROMagar™ Candida) from Mast Diagnostic, UK. Both media were prepared according to the manufacturers' instructions. Sabouraud agar (SAB+C) was supplied as ready-to-use agar plates in packs of 15 plates. Cornmeal agar (CMA) was sup-

Table 1 **Reference yeast strains used in this study**

Serial no.	Organism	Identification no.
1	*Candida albicans*	(324/94RA), (WK1), (122/94Rgl), (684/93), (455/94rgh), (455/4sm), (ATCC 3516), 01.544), (LSHTM3153), (91L)
2	*C. tropicalis*	(723/93L), (340SAB/94), (Y09.56)
3	*C. glabrata*	(644/94), (233/94), (100/94), (Y33.90)
4	*C. famata*	(421a), (421c)
5	*C. krusei*	(22/94), (Y02.48), (SU6)
6	*C. guilliermondii*	(Y02.02), (657b/93)
7	*C. kefyr*	(Y06.01)
8	*C. parapsilosis*	(937A0)
9	*C. dubliniensis*	(NCPF3108), (Brist)
10	*Saccharomyces cerevisiae*	(322/97T2), (322/97L2)

plied as prepared solid 100 mL medium in a flask. The agar was melted by heating and aliquoted into 5 sterile Petri dishes. The GT test was carried out using horse serum (TCS Biologicals Ltd, Buckingham, UK).

To assess the efficacy of CA medium as an isolation medium, all reference strains, stock cultures and clinical specimens were cultured on both SAB+C and CA agar in all phases of the study.

To evaluate the accuracy of CA medium as an identification medium, 30 reference yeast strains (Table 1) were used. Each specimen was plated onto quarter plates of SAB+C and CA media using a sterile loop. The inoculum was streaked out to obtain well-isolated colonies. The plates were incubated at 37 °C for 48 hours. Growth on both media, and colony colour and morphology on CA medium were recorded at 24 hours and 48 hours. Thereafter, all strains grown on SAB+C were identified using the conventional methods of GT test and culture on CMA medium, testing for chlamydospores (CHL), arthrospores (AR) and mycelia or pseudomycelia (MY)/(PMY). GT test-negative and *C. dubliniensis* strains were further investigated using the API 20 C AUX system (BioMérieux, Maray-l'Etoile, France). On CA agar, identity was assigned according to the manufacturer's guidelines and the published criteria of Odds and Bernaerts. The plates were stored at room temperature for future reference. Culture on CA medium was performed in duplicate using two different commercial versions of CA to detect any discrepancies between different makes of the medium.

The ability of CA medium to detect and identify yeast isolates from clinical specimens was examined. Urine specimens (n = 90) were well mixed and a standard inoculum of 1 mL inoculated onto quarter plates of SAB+C and CA media. The inoculum was streaked out to obtain single colonies. A drop of the blood culture specimen and 66 genital swab specimens were swabbed onto a quarter plate of SAB+C and CA media; the inoculum was streaked to single colonies. The plates were incubated aerobically at 37 °C for 48 hours.

The growth of urine specimens was scored quantitatively as colony forming units per mL (cfu/mL), while that of genital and blood specimens was scored quantitatively as negative (no growth), poor growth (< 100 colonies), moderate (100–1000 colonies) or heavy growth (> 1000 colonies).

The GT test and culture on CMA medium were performed using cultures grown on SAB+C media. All GT test-negative iso-

lates were subjected to further identification by an AUXACOLOR (Sanofi Diagnostics Pasteur, Marnes La Coquette, France) kit using an inoculum from SAB+C medium. Colony colour and morphology on CA medium were recorded and identity was determined according to the identification photograph supplied with the medium and the criteria of Odds and Bernaerts.

To test whether an inoculum from CA medium could be used for direct biochemical identification, 14 known isolates were reidentified using an inoculum taken from the CA plates instead of the recommended inoculum from Sabouraud agar.

The cost of and time required for full identification to the species level were recorded both for the conventional, rapid identification and identification using CA medium. Consumables were costed at their commercial list price. The cost of agar plates was calculated from the number of plates prepared from each pack, assuming that each plate holds 20 mL of prepared medium. Labour time per assay was calculated in Welcan units (WU) and the true cost assessed at the midpoint of the medical laboratory scientific officers (MLSO) grade 1 scale (£15 000 per annum or £0.14076 per minute). The cost of consumables plus labour from the point of primary isolation to complete identification to the species level was calculated. Overheads were not included. Time to complete identification was estimated in days. Time and cost per assay were assessed for three methods of identification.

Results

The ability of the CA medium to detect yeast was investigated in all phases of this study. CA medium allowed the 30 reference yeast strains to grow to the same extent as the SAB+C media. In the second phase using clinical specimens, 18 specimens showed no growth on either medium. One specimen was negative on SAB+C alone. One genital specimen showed heavy growth on CA medium but poor growth on SAB+C, and another genital specimen showed poor growth on CA and heavy growth on SAB+C. With 4 specimens, equal growth was detected on both media only after prolonged incubation at room temperature. In the remaining specimens, the extent of growth after 48 hours on both media was the same. Of the 140 positive specimens grown on CA, 8 were mixed cultures of 2 types of yeast and 1 was a mixed culture of 3 types, easily recognized by the colony appearance.

In the third phase, stock *C. albicans* strains (n = 105) were cultured on both CA and SAB+C media. In all, 6 strains failed to grow on either medium, 1 specimen showed mould contamination, and the rest grew equally well on both media. No bacterial contamination was observed on either medium at any point of the study. On identification media, all strains of *C. albicans* (n = 10) grew well, forming smooth, entire colonies with different degrees of colour intensity which ranged from light to dark green. *C. tropicalis* (n = 3) showed dark grey-blue colonies surrounded by a dark brownish-blue halo in the agar. *C. glabrata* strains (n = 4) gave pink to dark pink colonies with lighter edges. *C. famata* (n = 2) formed white colonies and *C. krusei* (n = 3) formed flat rough spreading pink colonies with white edges. *C. dubliniensis* (n = 2) formed green or dark green colonies similar to those of *C. albicans*. The remaining reference strains showed a variety of colours ranging from white to shades of pink. CA medium correctly identified all isolates of *C. albicans*, *C. tropicalis* and *C. krusei*. Isolates of *C. glabrata*, *C. famata*, *C. guil-*

ltermondii, *C. kefyr* and *C. parapsilosis* were collectively identified as *Candida* species. CA medium identified the 2 isolates of *C. dubliniensis* as *C. albicans*.

The conventional method of using GT test and CHL formation identified 9/10 of *C. albicans*, and identified *C. dubliniensis* as *C. albicans*, which was also misidentified by the AUXACOLOR and API 20 C systems. The API system correctly identified the single GT-negative *C. albicans* and the rest of the GT-negative *Candida* species.

On SAB+C medium, 139/158 clinical specimens were positive for yeast growth, 5 of which were recognized as mixed cultures. The total number of yeast isolates was 144. The conventional method identified 98 *C. albicans* and 46 *Candida* spp.; the AUXACOLOR system for GT-negative specimens (n = 46) identified *C. albicans* (n = 3), *C. glabrata* (n = 20), *C. tropicalis* (n = 5), *C. parapsilosis* (n = 7), *C. famata* (n = 2), *Rhodotorut rubra* (n = 4) and *C. krusei* (n = 1) and failed to identify 4 isolates.

Table 2 Recovery and identification of 158 clinical specimens on SAB+C and CA media

Isolate	SAB+C	CA
Candida albicans	101	102
C. tropicalis	5	7
C. krusei	1	1
C. glabrata[a]	20	
C. parapsilosis[a]	7	
C. famata[a]	2	
Rhodotorula rubra[a]	4	
Candida spp.[a]	4	39

[a] *Collectively identified as* Candida *spp. on CA medium.*
SAB+C = Sabourand agar.
CA = CHROMagar™ Candida.

On CA medium 140/158 specimens were positive, 8 of which were easily recognized as mixed cultures of 2 organisms, and 1 specimen as a mixed culture of 3 types of organism. The isolates were identified as *C. albicans* (n = 102), *C. tropicalis* (n = 7), *C. krusei* (n = 1) and *Candida* spp. (n = 39).

A summary of the results on the two media is given in Table 2.

Using 9 *C. albicans*, 1 *C. tropicalis*, 1 *C. krusei*, 1 *C. parapsilosis* and 2 *C. glabrata* samples, inocula were taken directly from CA medium and identified by conventional and AUXACOLOR methods. No difference was observed between the results using inocula direct from CA or from SAB+C media.

To analyse the cost of identification of 158 clinical specimens studied, different pathways were used. First, the specimens were inoculated onto quarter plates of SAB+C agar and identification of positive cultures was carried out using the GT test and the AUXACOLOR system (Figure 1). A cost analysis of using CA medium was carried out in two ways. In the first, all colonies identified as *Candida* spp. were subcultured on SAB+C agar, and identification was performed by the AUXACOLOR system using the standard inoculum from SAB+C according to the manufacturer's recommendation (Figure 2). In the second, inocula were taken directly from fresh 48-hour cultures on CA medium (Figure 3).

Pathway 1 showed a minimum time for identification of 6 days, and the cost per test following this pathway was calculated at £1.93. Pathway 2 required 6 days for identification and the cost was calculated at £1.42. Pathway 3 identified to the species level at day 2 for *C. albicans*, *C. tropicalis* and *C. krusei*, and at day 5 for other *Candida* species. This pathway was the shortest (5 days) and the cost of the test was calculated at £1.36.

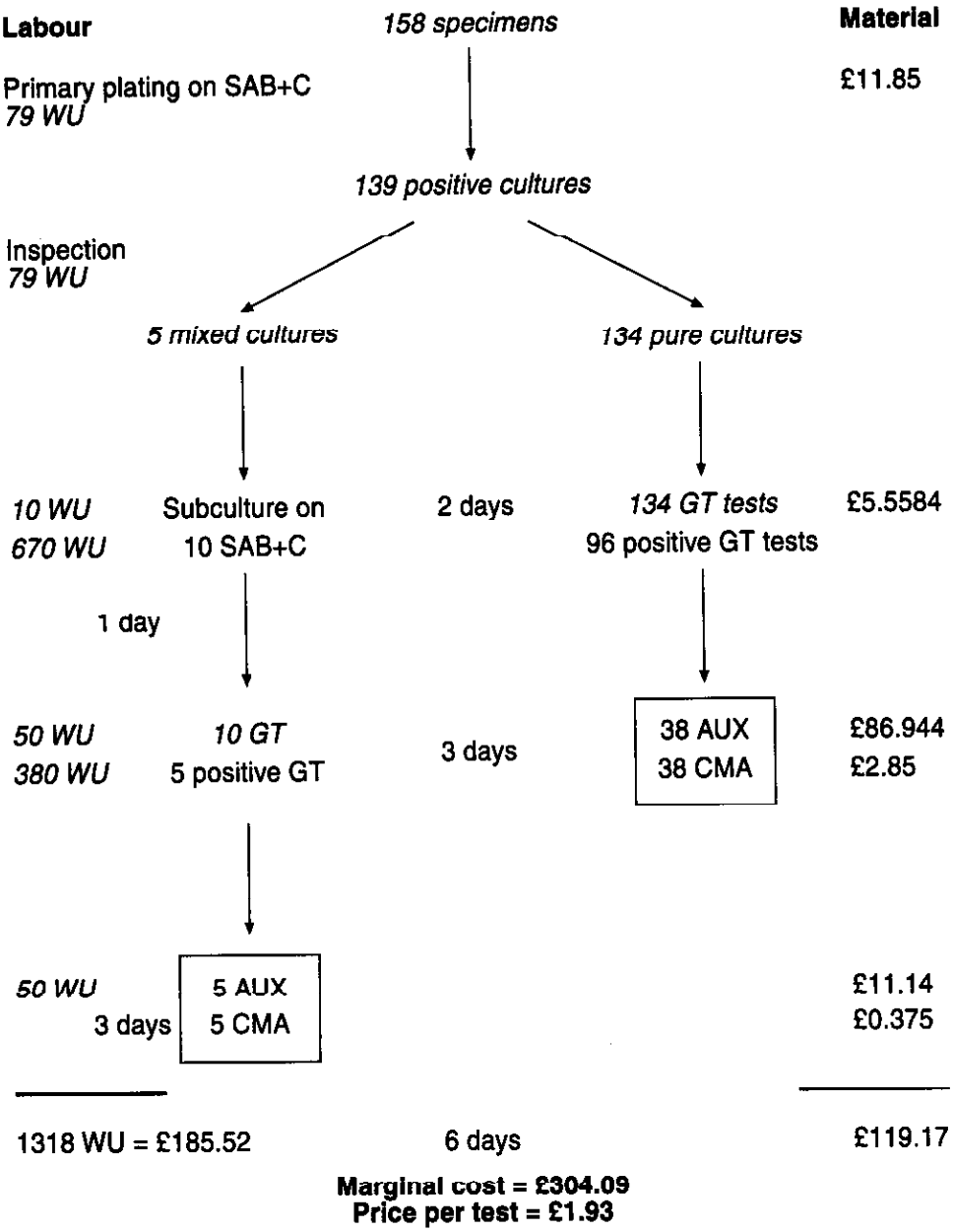

Figure 1 **Identification and costing of 158 clinical specimens, using a combination of the conventional and AUXACOLOR syeteme: pathway 1 (coste in pounds sterling)**

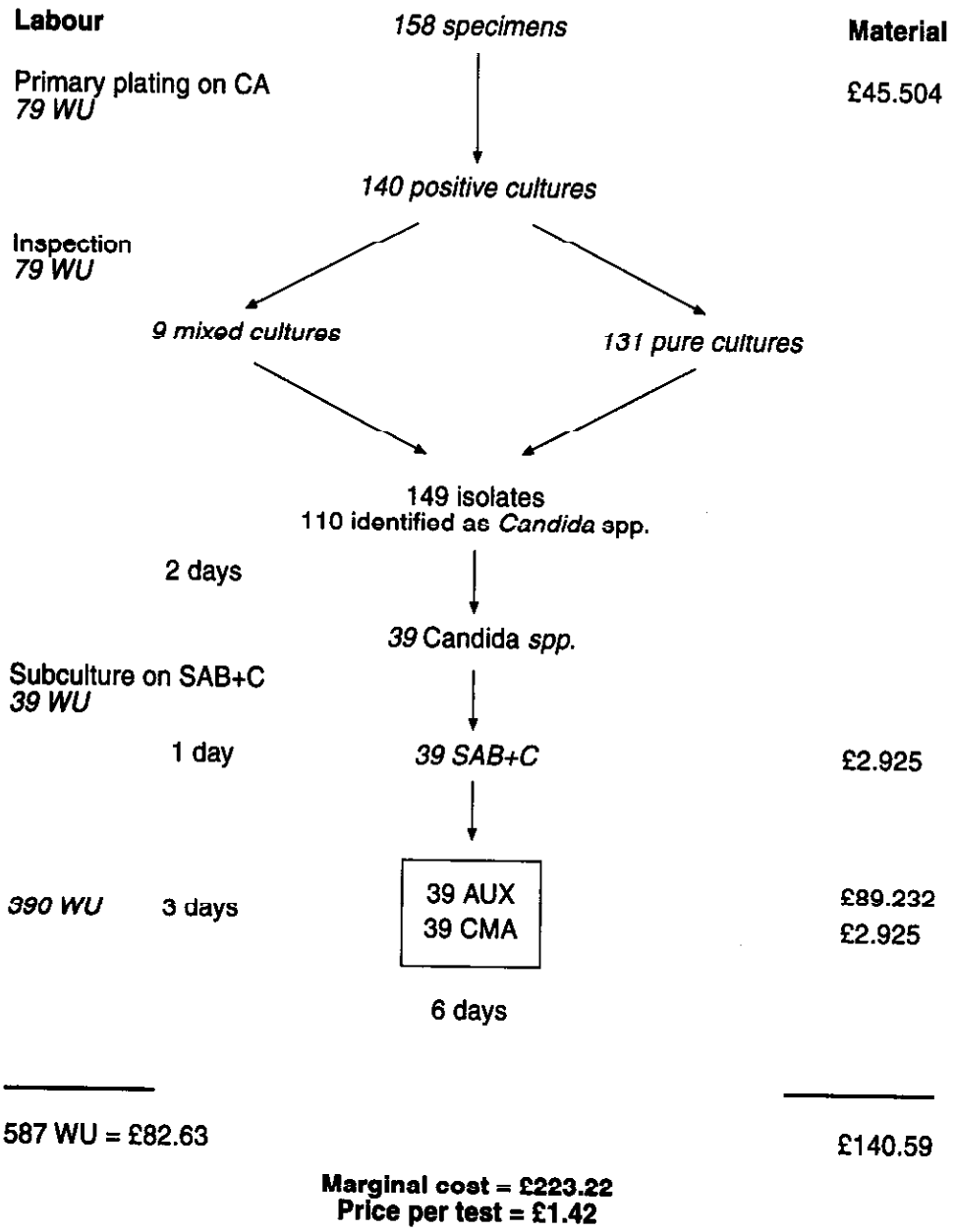

Figure 2 Identification and costing of 158 clinical specimens, using CA and SAB+C media: pathway 2 (costs in pounds sterling)

Discussion and conclusion

Although representing general categories of patients, the clinical materials used in this study were mainly genital and urine specimens. Other evaluations have focused on specific categories of patients [*8,10,13*]. The CA medium was found to be highly selective for yeasts, and no bacterial contamination was noticed in the study despite the fact that most of the specimens were taken from non-sterile body sites. As an isolation medium, the ability of CA medium to support the growth of *Candida* species is not significantly different from the widely used and most popular Sabouraud agar. After 24 hours incubation, growth was detected, but was not enough to allow differentiation and identification of the species.

Consistent with the finding of Odds and Bernaerts, there was no significant difference between the media in recovery rates on primary culture of the clinical specimens. A total of 149 isolates was grown on CA medium and 145 isolates on SAB+C. This gives a detection rate 3% higher on

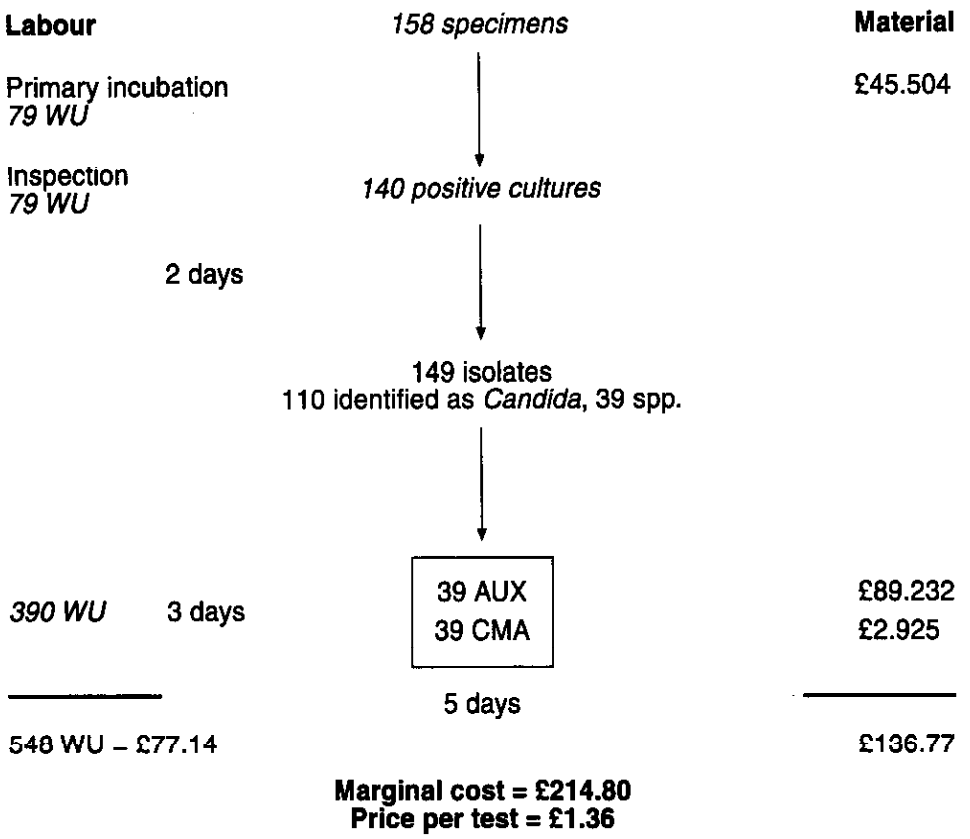

Figure 3 **Identification and costing of 158 clinical specimens, using CA medium and the AUXACOLOR system: pathway 3 (costs in pounds sterling)**

CA than on SAB+C. Higher sensitivity has been reported by several authors. Baumgartner [7] reported a 20% higher sensitivity on CA medium than on Sabouraud agar. Other studies [8,12,13,14] showed that CA was more sensitive in detecting *Candida* isolates than SAB+C.

The ability of CA medium to detect polymicrobial cultures is advantageous. Only 5 cultures on SAB+C were initially recognized (with great difficulty) as mixed cultures of 2 strains. Colony appearance on CA medium allowed easy recognition of 9 mixed cultures, 8 containing 2 types and 1 containing 3 types of yeast, resulting in the isolation of 19 strains. In contrast, on SAB+C, only 5 cultures could be recognized as mixed cultures of 10 isolates. Difficulty in recognizing mixed cultures on SAB+C can lead to misleading results when attempting identification.

The differential colony appearance on CA medium makes it extremely useful in making a rapid presumptive identification of the most common *Candida* species. As reported originally by Odds and Bernaerts, the green colour is unique to *C. albicans*. Casal [15] described dark green colonies as characteristic of *C. zeylanoides*, a species not mentioned in the study of Odds and Bernaerts. In this study, *C. dubliniensis* showed the same colour as *C. albicans*. However, in older cultures the colour is less intense while in freshly cultured clinical specimens, it appears as dark green. This phenomenon was described by Sullivan et al. [16] as a way of differentiation between the two species on primary culture. Our results were consistent with these findings as 2 stock cultures and 1 reference strain appeared as light green, while the other fresh reference strain and 2 clinical isolates appeared dark green on CA medium.

C. albicans isolated from clinical specimens represented 69% of the total number of isolates. CA medium correctly identified 100% of strains previously identified by reference methods (GT test, CHL production and AUXACOLOR system). The GT test was found to have low sensitivity and specificity. Three isolates were negative by the GT test and were identified by the AUXACOLOR system as *C. albicans*. The production of germ tube-like structures by *C. tropicalis* may give misleading results. Formation of chlamydospores, structures specifically produced by *C. albicans*, was not detected in 9% of the *C. albicans* isolates. The presence of dark blue colonies with dark bluish or brownish haloes in the agar, characteristic of *C. tropicalis*, allowed 7 isolates of *C. tropicalis* to be detected, while only 5 were isolated from SAB+C medium. The higher sensitivity of CA medium in detecting *C. tropicalis* has previously been reported by Pfaller et al. [12]. *C. parapsilosis*, a pathogen recovered mostly from the hands of nurses [17], was detected in 7 instances as white colonies. Casal et al. [15] reported that the white colony colour on CA medium is useful for the presumptive identification of *C. parapsilosis* and the pathogenic algae *Prototheca* spp.

Overall, the accuracy of CA medium in this study was consistent with the results of Odds and Bernaerts in presumptive identification of *C. albicans*, *C. tropicalis* and *C. krusei* on the basis of colony appearance. In contrast to previous reports, *C. glabrata* could not be correctly identified [12]. The variation in pink colour in these isolates was too wide to allow accurate identification.

Odds and Bernaerts found that CA medium did not affect the viability of the yeast inocula. Similarly, Pfaller found that using inocula directly from CA medium had no adverse effects on sensitivity or confirmatory tests. This study confirms these find-

ings. Results of identification with the AUXACOLOR system using inocula directly from CA medium gave the same biochemical profile and morphology as inocula obtained from SAB+C. These findings demonstrate the suitability of using CA for confirmatory or supplementary testing without time-consuming subculture on SAB+C. CA medium can also be used at wide temperature ranges. Left at room temperature, 4 isolates of *R. rubra* grew well and to the same extent as on SAB+C. The orange colour of the isolates was not affected. Using the medium at 45 °C allowed the growth and identification of *C. albicans* strains.

No differences in the test results were noted when two different commercial versions of CA were used.

Clinical laboratories usually follow identification protocols that vary from one laboratory to another, depending on the workload. Using CA medium, identification to the species level from the point of primary isolation was achieved 3 days earlier for *C. albicans*, *C. tropicalis* and *C. krusei* and 1 day earlier for other *Candida* species than when using conventional methods, which allows earlier selection of appropriate antifungal therapy. Savings in labour and time were also gained by the use of CA medium: identification took 40% of the time required by conventional methods (584 WU versus 1318 WU). The extra time available could be used to cope with the increasing laboratory workload. Although the price of CA medium was four times higher than that of SAB+C, the final cost of the test is far less than the cost when conventional methods are used (£1.36 versus £1.93 per test).

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