

Correspondence

Evaluation of CHROMagar candida for rapid identification and Etest for antifungal susceptibility testing in a district general hospital laboratory

The incidence and clinical importance of fungal infections in immunocompromised patients is increasing, and the isolation of multiple yeast species from clinical specimens is not uncommon. *Candida albicans* remains the most frequently isolated yeast species; but others, inherently or potentially resistant to amphotericin B and azole compounds (*C krusei*, *C parapsilosis*, *C lusitanae*, *C tropicalis*, and *C glabrata*), are also being reported.¹

Unlike sabouraud dextrose agar (SDA) (LabM, Bury, UK), CHROMagar candida (Mast Diagnostics, Bootle, UK), a chromogenic, differential culture medium, can detect mixed populations and facilitate rapid, accurate identification of *C albicans*.²

The procedure for antifungal susceptibility testing by the broth based NCCLS reference method M27-A is time consuming.³ The Etest (AB BIODISK, Solna, Sweden), a simple alternative agar based quantitative diffusion method, can be readily incorporated into a clinical laboratory routine.⁴

Over a period of 10 weeks, 31 yeast isolates were recovered on SDA, from 22 patients in intensive care, HIV, and Oncology/Haematology units of the North Middlesex Hospital NHS Trust. Colony pigmentation, following subculture on to CHROMagar candida, was assessed after 48 hours incubation at 37°C. Two specimens yielded mixed populations. The identification of the isolates was confirmed using API 20C AUX (Bio Mérieux, Basingstoke, UK).

Twenty five isolates, yielding green colonies, were identified as *C albicans* (germ tube positive). Six isolates, yielding non-green colonies and germ tube negative, were identified as *C glabrata* (four), *C parapsilosis* (one), and *C norvegensis* (one; unidentified by API 20C AUX, but confirmed by Centraalbureau voor Schimmelcultures, Delft).

Amphotericin B, fluconazole, and itraconazole MICs were determined by our laboratory (laboratory 1) using the Etest on RPMI 1640 containing 2% glucose and MOPS buffer (RPMI) and casitone agar (Cambridge Diagnostic Services Ltd, Cambridge, UK). The performance of commercially bought agar (RPMI and casitone) was within the recommended specifications. MIC endpoints were read after 24, 48, and 72 hours incubation at 37°C, and adjusted to the next upper twofold value if MICs evaluated by Etest



Figure 1 Fluconazole (FL): change of morphology at endpoint. MIC, 48 mg/litre; itraconazole (IT): sharp end point. MIC, 0.5 mg/litre.

were in between dilutions. Etest reproducibility was examined by another laboratory (laboratory 2; AB BIODISK, Solna, Sweden) and MICs correlated with semi-automated spectrophotometric readings of microdilution broth cultures by a third laboratory (laboratory 3; Janssen Research Laboratory, Beerse, Belgium).⁵ The quality control strains used by laboratory 1 were *C albicans* ATCC 90028 and *C glabrata* ATCC 90030; laboratories 2 and 3 used *C parapsilosis* ATCC 22019 and *C krusei* ATCC 6258 as controls. The results obtained with these strains were within the expected limits; the exception was itraconazole, which was higher by one dilution in laboratory 1 and lower by one dilution in laboratory 3. The MIC ranges (mg/litre) defined by NCCLS⁵ and recommended by AB BIO DISK (shown in parenthesis) are as follows: *C parapsilosis* ATCC 22019 were 0.25 to 1 (2), 2 to 8 (16), and 0.06 to 0.25; *C krusei* ATCC 6258 were 0.5 to 2, 16 to 64 (≥ 256), and 0.12 to 0.5; *C albicans* ATCC 90028 were 0.25 to 2, 0.25 to 1, and ≤ 0.125 to 0.5; and *C glabrata* ATCC 90030 were 0.5 to 2, 8 to ≥ 32 , and 0.5 to ≥ 32 for amphotericin B, fluconazole, and itraconazole, respectively.

Interlaboratory reproducibility (results within two doubling dilution difference) of

Etest MICs, determined after 48 hours on RPMI and casitone agar, was 100% and 97% (amphotericin B), 97% and 90% (fluconazole), and 90% and 87% (itraconazole), respectively. The correlation between the Etest MICs using RPMI and casitone agar at 48 hours and the spectrophotometric method was 94% and 77% (amphotericin B), 100% and 65% (fluconazole), and 32% and 13% (itraconazole), respectively. Etest MICs on RPMI were more reproducible and correlated better with the spectrophotometric method than those on casitone agar (table 1). Etest MICs for itraconazole were greater than those obtained by the spectrophotometric method. Trailing endpoints (fig 1) did not hinder the interpretation of the fluconazole Etests, and isolates exhibiting itraconazole resistance by the Etest were not all confirmed by the spectrophotometric method. The itraconazole discrepancy between Etests and broth spectrophotometry is high, and further work is needed to obtain a better correlation. The correct selection of MIC end points is crucial, and might have had an impact on the results.

The rapid identification and accurate susceptibility testing of yeast would help to modify treatment and influence clinical outcome. We conclude that CHROMagar candida and Etest are worth considering in a district general hospital setting.

Table 1 MIC ranges for yeast isolates as determined by Etest and spectrophotometric broth microdilution methods

Antifungals	Methods	MIC range (mg/litre)	MIC ₅₀ (mg/litre)	MIC ₉₀ (mg/litre)
Amphotericin B	Etest RPMI/casitone (lab 1)	0.125 to 1/0.03 to 0.25	0.25/0.125	0.5/0.125
	Etest RPMI/casitone (lab 2)	0.125 to 1/0.06 to 0.5	0.125/0.125	0.5/0.25
	Broth microdilution (lab 3)	0.06 to ≥ 4	0.25	0.5
Fluconazole	Etest RPMI/casitone (lab 1)	0.125 to 64/0.5 to 64	0.25/2	32/8
	Etest RPMI/casitone (lab 2)	0.25 to ≥ 256 /1 to ≥ 256	0.5/2	32/64
	Broth microdilution (lab 3)	0.125 to 64	0.25	32
Itraconazole	Etest RPMI/casitone (lab 1)	0.03 to ≥ 32 /0.06 to ≥ 32	0.25/0.25	≥ 32 /8
	Etest RPMI/casitone (lab 2)	0.03 to ≥ 32 /0.06 to ≥ 32	0.25/0.125	≥ 32 / ≥ 32
	Broth microdilution (lab 3)	0.008 to 2	0.008	1

We would like to thank Å Karlsson (laboratory 2) and F Odds (laboratory 3) for their valuable comments and for performing the susceptibility testing.

J E AMBLER
M KERAWALA
A YANEZA
Y J DRABU
Department of Microbiology,
North Middlesex Hospital NHS Trust,
London N18 1QX, UK

1 Jarvis WR. Epidemiology of nosocomial fungal infections, with emphasis on candida species. *Clin Infect Dis* 1995;20:1526-30.

- 2 Pfaller MA, Houston A, Coffman S. Application of CHROMagar for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida (Torulopsis) glabrata*. *J Clin Microbiol* 1996;34:58-61.
- 3 National Committee for Clinical Laboratory Standards. *Reference method for broth dilution antifungal susceptibility testing for yeasts, 1997. Approved standard M27-A*. Villanova, Pennsylvania, USA: NCCLS.
- 4 Van Eldere J, Joosten L, Verhaeghe A, et al. Fluconazole and amphotericin B antifungal susceptibility testing by national committee for clinical laboratory standards broth macrodilution method compared with E-test and semi-automated broth dilution test. *J Clin Microbiol* 1996;34:842-7.
- 5 Odds FC, Vranckx L, Woestenborghs F. Antifungal susceptibility testing of yeasts: evaluation of technical variables for test automation. *Antimicrob Agents Chemother* 1995;39:2051-60.

The use of the Reflection computer program for facilitating report formulation in the medical microbiology laboratory

There are several different computer systems in use in UK medical microbiology laboratories. Rule bases can be built into the system for the automatic release of comments or antibiotic sensitivities on laboratory reports in predetermined situations to aid manual amendment. The time consuming nature of data entry has already been highlighted.¹ We describe a method to facilitate data entry and report amendment.

Within our hospital, all result entry and reporting is performed through the pathology management system APEX (ACT Medisys Ltd, Newbury, Berkshire, UK). Most of the data input on to this system is via dumb terminals that attach to the server through the hospital network. In addition, there are presently two PCs within the department also attached to the hospital network running either Windows 95 or NT. By utilising the terminal emulation software Reflection (WRQ/Wickhill, Guildford, Surrey, UK), these PCs are able to replicate all the functionality achieved from a terminal on a windows based PC platform. However, be-

cause Reflection is a software component, it can incorporate features unavailable to a terminal.

A toolbar programme within the Reflection programme can be used to facilitate report amendment. This enables a toolbar to be created with icons on which a frequently used string of keystrokes can be recorded then played back by clicking on the icon. The computer can therefore be used like a tape recorder by recording keystrokes instead of sound. Figure 1 demonstrates the creation of an icon.

The method can be used to assist various functions, such as comment entry or amending antibiotic sensitivities. For example, to enter a comment:

- Icon 1 = to arrive at the comment entry point = five keystrokes.
- Icon 2 = comment "contaminated urine sample, please send a repeat" = 44 keystrokes.
- Icon 3 = to return to the main menu = five key strokes.

In this example 54 keystrokes have been reduced to three.

Different toolbars can be created for each group of samples—for example, sputum, urine, etc.

Figure 1 shows three toolbars open simultaneously, thus allowing toolbars to be used together. Customised toolbars can be saved on to disc, allowing them to be used on different machines, or for different users to have their own customised disc.

An important feature of a UK microbiology laboratory is the input of the medical microbiologist in laboratory supervision and report authorisation. Report amendment can be time consuming but can make a report more user friendly for the clinician and facilitate clinical care. The toolbar programme can assist this process.

The program can also be used by technical staff. A further advantage of this program is that it can be used as an adjunct to most computer systems. A hospital in which there is an integrated network can therefore incor-

porate the use of Reflection without having to change its current computer system.

This program may assist in more effective time management for hospital microbiologists, an increasingly important factor if their time is to be deployed in the most effective way.

The Reflection package is produced by WRQ (UK distributors: Wickhill Ltd, Bradstone Brook, Christmas Hill, Shalford, Guildford, Surrey GU4 8HR. Tel: +44 01483 466 500).

M VIAGAPPAN

Microbiology Department, University Hospital
Lewisham, Lewisham High Street, London
SE13 6LH, UK

S HOWARD

Business Development, 2nd Floor Mulberry House,
Royal Marsden Hospital, Fulham Road, London
SW3 6JJ, UK

- 1 Block C. Benefits and limitations of computerised laboratory data. *J Clin Pathol* 1997;50:448-9.

Book reviews

Diagnostic Pathology of Parasitic Infections with Clinical Correlations.

Gutierrez Y. (£125.00.) Oxford University Press, 2000. ISBN 0 19 512143 0

The determination of parasites in tissue sections is generally regarded as one of the most difficult aspects of the diagnosis of parasitic infections. Very few good books about this topic are available, which makes *Diagnostic Pathology of Parasitic Infections* by Yezid Gutierrez most welcome.

This impressive book of 769 pages includes, according to the author, all parasites described until now in human tissues. The book is intended as a diagnostic tool for anatomical pathologists confronted with unknown parasites in tissue sections. Because of this orientation, aspects dealing with diagnosis in the clinical laboratory are only briefly mentioned. Morphological features of most of the protists, nematodes, trematodes, cestodes, and arthropods are discussed extensively in the text and illustrated with high quality (black and white) photographs and diagrams. In addition, several diagnostic stages are illustrated within 16 colour plates in the middle section of the book.

What makes the book of special interest is the link between the pathology observed in the tissues and the (extensive) description of clinical symptomatology of the infection. In this way a unique insight into the different host-parasite interactions is given.

In general, the chapters are well written. However, in this 2000 edition, the literature cited in some sections is a little outdated (most of the more recent references are from 1996 to 1997, with only a few in 1998). Nonetheless, when a good book about the diagnosis of parasitic stages in tissue samples and extensive clinical information of parasitic the infection is wanted, this book can be recommended.

T VAN GOOL

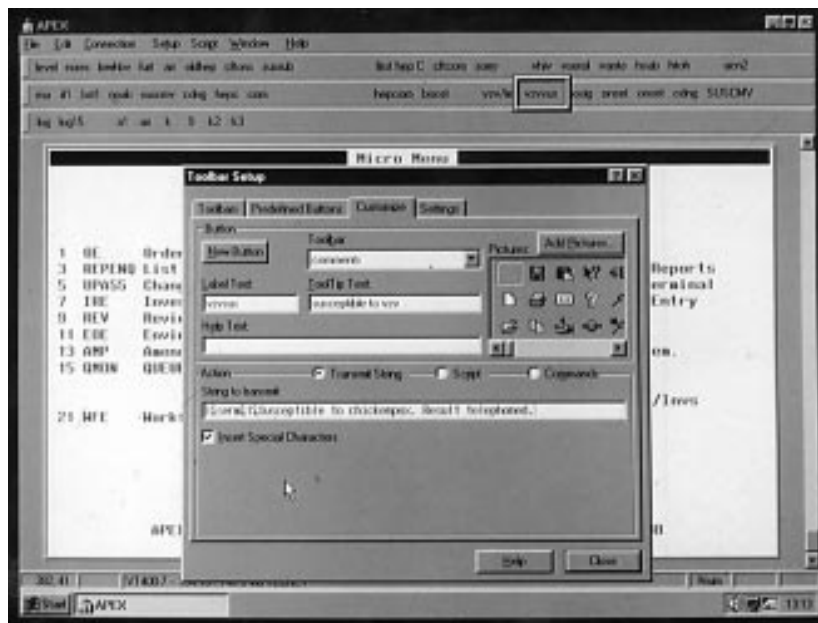


Figure 1 The Reflection toolbar program has the facility to record both text and function keystrokes and allow these to be played back. The "string to transmit" box shows the letters and function keys being recorded.

Biopsy Interpretation of Bone and Bone Marrow: Histology and Immunohistology in Paraffin and Plastic, 2nd ed. B Frisch, R Bartl. (£98.50.) Arnold (co-published with Oxford University Press in USA), 1999. ISBN 0 340 74089 2.

Biopsy Interpretation of Bone and Bone Marrow is a second, completely revised edition of a similar book published in 1985 by the same authors, but the book also resembles the *Atlas of Bone Marrow Pathology* published in 1990 by these authors.

In 31 chapters, the authors give a comprehensive overview of the diagnostic features of most disorders of the bone and bone marrow. After four introductory chapters, nine chapters are entirely dedicated to bone diseases. In chapter 14, metastatic bone disease is described, and in the following chapters most bone marrow disorders including the lymphoproliferative disorders are described. Sometimes, an introductory chapter precedes more specific chapters.

The book contains highly valuable information on issues that are not easily found in other text books, especially on bone diseases. It reflects the enormous experience of both authors in this field of diagnostic pathology.

In general, each chapter is clearly written and contains comprehensive tables either listing general features of a specific disorder, clinical characteristics, histological characteristics, or checklists.

The layout is excellent and the illustrations (most representing Giemsa stained slides of plastic embedded biopsies) with the schematic drawings are really superb.

However, there is also some criticism possible: in general, the background information (especially on pathophysiology) for each disorder is limited and the text is often relatively superficial. Just to give one example, in chapter 18, brief information on the different types of haemolytic disorders should be given, at least to alert the pathologist to the possibility of detecting a non-Hodgkin's lymphoma in the biopsy in association with cold agglutinin disease.

In some chapters, the data are incorrect. For instance, in the lymphoma chapters 25 and 27, data on the localisation of specific subtypes of non-Hodgkin's lymphomas are given. According to these data mantle cell lymphoma has a preferential paratrabeular localisation. This is incorrect, the infiltrates in mantle cell lymphoma being patchy and entirely random. It is likely that the described paratrabeular infiltrates represent localisation of follicle centre cell lymphomas with very few centroblasts often mixed up with centrocytic lymphoma in the past, but now very easy to distinguish with immunohistochemistry. An amusing error is shown in tables 25.6 and 25.7 because the REAL and ILSG classifications are essentially the same, the author probably referring to the novel WHO classification in table 25.7. An insufficient updating in this area is also illustrated by the absence of more recently described entities such as hepatosplenic $\gamma\delta$ T cell

lymphoma with a diagnostic intrasinusoidal pattern of neoplastic T cells, best visible in bone marrow biopsies. In these chapters on lymphomas many other (small) errors, also concerning the cytogenetic data, can be found.

One disturbing general feature of the book is the lack of specific references in the text, whereas the references are listed alphabetically after each chapter. This makes it almost impossible to go back to the original literature data. A related weakness is the lack of quotation of sources in cases where detailed numerical data are given. For instance, in chapter 14 metastatic bone disease is described and data on the incidence of metastasis in breast cancer, prostate cancer, etc, are given. These data seem to be derived from the authors' large collections, but it is unclear how these series were selected (for instance, an incidence of 42% positive bone marrow biopsies in a random series of patients with breast cancer is very high and suggests that this represented a selected series of patients with advanced disease).

In summary, this book is an excellent atlas of bone and bone marrow pathology and as such it is an important adjunct for each (haemato)pathologist. However, it is of limited value to obtain adequate (background) information.

P KLUIN

Further details: The Academic Secretary, Department of Haematology, St Mary's Hospital Campus of ICSM, Norfolk Place, London W2 1PG, UK. (Fax +44 020 7262 5418)

Histopathology of the Bone Marrow

28 March 2001, St Mary's Hospital, London, UK

Further details: The Academic Secretary, Department of Haematology, St Mary's Hospital Campus of ICSM, Norfolk Place, London W2 1PG, UK. (Fax +44 020 7262 5418)

Haematological Morphology and Leukaemia Classification for Cytogeneticists

29 March 2001, St Mary's Hospital, London, UK

Further details: The Academic Secretary, Department of Haematology, St Mary's Hospital Campus of ICSM, Norfolk Place, London W2 1PG, UK. (Fax +44 020 7262 5418)

6th European Forum on Quality Improvement in Health Care

29–31 March 2001, Bologna, Italy

Further details: BMA/BMJ Conference Unit, BMA House, Tavistock Square, London WC1H 9JR, UK. (Tel +44 020 7383 6409; fax +44 020 7383 6869; email Quality@bma.org.uk; website www.quality.bmj.com)

BSCC London Spring Tutorial: Lung and Pleural Cavity Fluid Cytology

27 April 2001, Guy's Hospital, London, UK

Further details: BSCC Office, PO Box 352, Uxbridge UB10 9TX, UK. (Tel +44 01895 274 020; fax +44 01895 274 080; email lesley.couch@psilink.co.uk)

International Consultation on the Diagnosis of Noninvasive Urothelial Neoplasms

11–12 May 2001, University of Ancona School of Medicine, Torrette, Ancona, Italy

Further details: R Montironi, Ancona Italy (email r.montironi@popcsi.unian.it), DG Bostwick, Richmond, VA, USA (email bostwick@bostwicklaboratories.com), P-F Bassi, Padua, Italy (email bassipf@ux1.unipd.it), M Droller, New York, USA (email michael_droller@smtplink.mssm.edu), or D Waters, Seattle, WA, USA (email waters@vet.vet.purdue.edu)

Calendar of events

Full details of events to be included should be sent to Maggie Butler, Technical Editor JCP, The Cedars, 36 Queen Street, Castle Heddingham, Essex CO9 3HA, UK; email: maggiebutler@pilotree.prestel.co.uk

BSCC Northern Spring Tutorial: Gynaecological Cytology

8 March 2001, Manchester, UK
Further details: BSCC Office, PO Box 352, Uxbridge UB10 9TX, UK. (Tel +44 01895 274 020; fax +44 01895 274 080; email lesley.couch@psilink.co.uk)

Urological Surgical Pathology for the Practising Pathologist

24–26 March 2001, Sanibel Harbour Resort and Spa, Fort Myers, Florida, USA
Further details: Department of Continuing Education, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA. (Tel +1 617 432 1525; fax +1 617 432 1562; email hms-cme@hms.harvard.edu)

Haematology Morphology

26–27 March 2001, St Mary's Hospital, London, UK



Evaluation of CHROMagar candida for rapid identification and Etest for antifungal susceptibility testing in a district general hospital laboratory

J E Ambler, M Kerawala, A Yaneza, et al.

J Clin Pathol 2001 54: 158-159

doi: 10.1136/jcp.54.2.158

Updated information and services can be found at:

<http://jcp.bmj.com/content/54/2/158.full.html>

These include:

References

This article cites 4 articles, 4 of which can be accessed free at:

<http://jcp.bmj.com/content/54/2/158.full.html#ref-list-1>

Article cited in:

<http://jcp.bmj.com/content/54/2/158.full.html#related-urls>

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>