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# Evaluation of a new selective chromogenic agar for detection of *Burkholderia cepacia* complex

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## Introduction

Species of the *Burkholderia cepacia* complex (Bcc) can infect lung of cystic fibrosis (CF) patients and are associated with a significant increase in morbidity and mortality. Early and precise detection of Bcc in respiratory specimens from people with CF is essential for appropriate patient treatment and management. The detection of Bcc is culture dependent, preferably using *B. cepacia* selective agar (BCSA) and species identification is based on MALDI–TOF MS combined with PCR-based assays.

A new selective chromogenic agar CHROMagar™ B.cepacia (CHROMBc) (CHROMagar, Paris, France) was evaluated in comparison to BCSA for the ability to grow Bcc and inhibit other organisms in medical microbiology laboratories supporting three CF centres in Argentina (Hospital de Niños Ricardo Gutierrez and Hospital de Pediatría Juan P. Garrahan from Buenos Aires, Hospital de Niños Sor María Ludovica from La Plata) and one in Slovenia (Universitiy Clinical Center Ljubljana).

## Materials and Methods

### *Media*

CHROMBc was provided to all laboratories as a dehydrated powder by the company CHROMagar (Paris, France).
Institute of Microbiology and Immunology, University of Ljubljana, Faculty of Medicine (lab A) and microbiology laboratory in Hospital de Niños Ricardo Gutierrez (lab B) used BCSA from Oxoid (Basingstoke, UK), while microbiology laboratories in Hospital de Pediatría Juan P. Garrahan (lab C) and Hospital de Niños Sor María Ludovica (lab D) used BCSA from Laboratorios Britania (Buenos Aires, Argentina).

### *Bacterial isolates*

In the first part of the evaluation 23 reference strains as well as 35 clinical isolates belonging to Bcc were used (Table 1).
CHROMBC and BCSA were inoculated with approximately $10^5$ CFU of each of the reference strain and incubated at 35 °C for 48 h.
35 clinical isolates of *Burkholderia* species including 26 *Burkholderia contaminans*, 5 *Burkholderia cenocepacia* and 4 *Burkholderia cepacia* were retrieved from the stored collection of the Argentinian laboratories (Table 1). These isolates were recovered in previous years from different CF patients. Clinical isolates were subcultured from a fresh overnight culture on blood agar directly onto CHROMBc and BCSA, without adjusting inoculum.

### *Culture of clinical samples*

In the second part of the evaluation, 304 prospective respiratory clinical samples (Table 2), mostly but not exclusively obtained from CF patients were inoculated on a standard set of media for CF specimens including BCSA and additionally on CHROMBc and cultured for a range of pathogens in line with routine laboratory procedures.
In the lab A 3 non-respiratory clinical samples (Table 2), were also included in the evaluation and inoculated on BCSA and CHROMBc immediately after Bcc species grew unexpectedly from these samples.
Inoculated CHROMBC and BCSA were incubated at 35 °C for 72 h and read daily.
Bacteria and yeasts recovered on all media were identified using matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS). MBT COMPASS 4.1, Microflex (Bruker Daltonics, Bremen, Germany) was used in lab A and VITEK MS (bioMerieux, Marcy l'Etoile, France) in labs B, C and D. PCR-based approach was used for final identification of the majority of Bcc species isolated from clinical samples.

## Results

### *Bacterial isolates*

All Bcc and *Burkholderia gladioli* reference strains as well as stored clinical isolates grew well on CHROMBc as characteristic green blue colonies some of them with halo (Figure 1 and Table 1).
Colonies were visible and fully developed after 24 h and 48 h, respectively.
*P. aeruginosa*, *K. pneumonia*, *S. aureus* and *S. epidermidis* reference strains didn't grow on CHROMBc.

Table 1. Reference strains included in the study (LMG - Laboratorium Microbiologie Ghent at the University of Ghent, Belgium)

| Species | Strains | Colony appearance after 48 h |
|---|---|---|
| *Burkholderia cepacia*: | ATCC 25416 | green blue with halo |
| | LMG 1222 | green blue with halo |
| | 4 clinical isolates | green blue colonies +/- halo |
| *Burkholderia multivorans*: | LMG 13010 | green blue with halo |
| | LMG 16660 | green blue colonies +/- halo |
| | LMG 16775 | green blue colonies +/- halo |
| *Burkholderia cenocepacia* IIIA: | LMG 12614 | green blue |
| | LMG 16656 | grey blue |
| | LMG 18863 | green blue colonies +/- halo |
| *Burkholderia cenocepacia* IIIB: | LMG 16654 | green blue |
| | LMG 18829 | green blue |
| | LMG 18830 | green blue |
| *Burkholderia cenocepacia* : | 5 clinical isolates | green blue colonies +/- halo |
| *Burkholderia stabilis*: | LMG 14294 | green blue |
| *Burkholderia vietnamiensis*: | LMG 18835 | green blue with halo |
| *Burkholderia dolosa*: | LMG 18943 | green blue with halo |
| *Burkholderia pyrrocinia*: | LMG 21824 | green blue with halo |
| *Burkholderia contaminans*: | LMG 23361 | green blue colonies +/- halo |
| | 26 clinical isolates | green blue colonies +/- halo |
| *Burkholderia gladioli*: | LMG 18157 | green blue |
| *Pseudomonas aeruginosa*: | ATCC 27853 | no growth |
| | ATCC 15442 | no growth |
| *Klebsiela pneumoniae*: | ATCC 700603 | no growth |
| *Staphylococcus aureus*: | ATCC 29213 | no growth |
| | ATCC 6538 | no growth |
| *Staphylococcus epidermidis*: | ATCC 14990 | no growth |

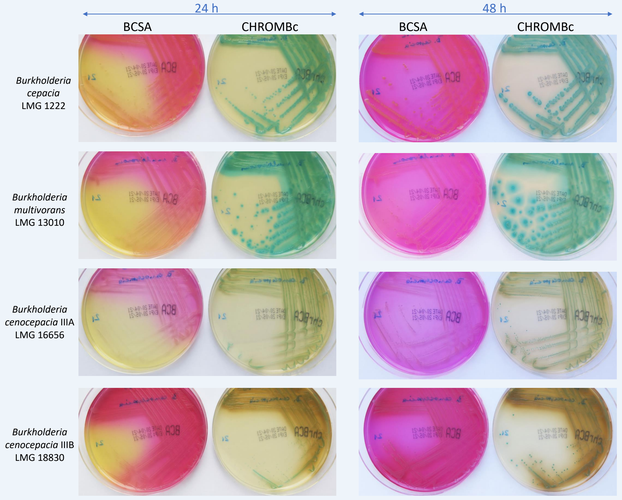


Figure 1. Colonies of 4 different Bcc reference strains on BCSA and CHROMBc after 24 h and 48 h of incubation.

### *Culture of clinical samples*

From 19 out of 307 clinical samples Bcc was isolated on both CHROMBc and BCSA (Table 2).
All Bcc isolates were visible on CHROMBc within the first 24 h.
One *B. cepacia* isolate and all nine *B. contaminans* isolates were obtained from Argentinian CF patients. Seven *B. cenocepacia*, and one each *B. cepacia* and *B. ambifaria* isolates were obtained from adult Slovenian non-CF patients. Seven of these 9 Slovenian patients were hospitalized in ICU.

Table 2. Clinical samples by type and Bcc positivity

| Sample type (No) | Bcc positive | Bcc species (No) |
|---|---|---|
| Sputum (172) | 10 | *B. contaminans* (8)<br>*B. cenocepacia* (1)<br>*B. cepacia* (1) |
| Throat aspirate (55) | 0 | - |
| Coughed throat swab (37) | 1 | *B. contaminans* |
| Tracheal aspirate (31) | 5 | *B. cenocepacia* (4)<br>*B. ambifaria* (1) |
| Bronchoalveolar lavage (6) | 0 | - |
| Nose swab (2) | 0 | - |
| Pus from maxillary sinus (1) | 0 | - |
| Blood culture (1) | 1 | *B. cenocepacia* |
| Cerebrospinal liquid (1) | 1 | *B. cepacia* |
| Urine (1) | 1 | *B. cenocepacia* |
| **Total (307)** | **19** | |

In rare cases non-Bcc species growth was observed on CHROMBc and/or BCSA plates inoculated with clinical samples. Details are presented in Table 3.
„Bcc-like" colony morphology of two non-Bcc species isolated from clinical samples on CHROMBc is shown in Figures 2 and 3.

Table 3. Growth of non-Bcc species observed on CHROMBc and BCSA plates inoculated with clinical samples. Numbers represent number of samples which resulted with non-Bcc growth.

| Microorganism | Growth on CHROMBc and BCSA (CHROMBc colony appearance) | Growth on CHROMBc only (colony appearance) | Growth on BCSA only |
|---|---|---|---|
| Yeast | 5 (white / blue / greenish) | 4 (white / blue / greenish) | 2 |
| *Pseudomonas aeruginosa* | - | 5 (transparent) | - |
| *Sphyngomonas paucimobilis* | - | 1 (yellow + green halo) | - |
| *Chryseobacterium indologenes* | - | 1 (yellow) | - |
| *Pandorea pnomenusa* | 1 (transparent) | - | - |
| *Achromobacter sp.* | 1 (transparent) | - | 1 |
| *Proteus mirabilis* | - | - | 1 |
| *Sphingobacterium sp.* | - | - | 1 |
| *Aspergillus fumigatus* | 2 (mold) | 1 | 1 |
| *Scedosporium sp.* | 1 (mold) | - | 1 |

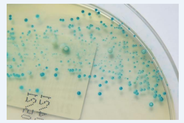

Figure 2. Colonies of yeasts isolated on CHROMBc form a sputum of a CF patient.

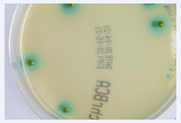

Figure 3. Colonies of *Sphyngomonas paucimobilis* isolated on CHROMBc form a throat aspirate of a pediatric CF patient.

## Conclusions

CHROMagar™ B.cepacia, a new selective chromogenic agar for detection of Bcc, enables rapid (< 48h) recovery of Bcc culture and inhibits a great majority of non-Bcc organisms.

Complementary methods have to be used for final genus and species level identification of colonies found on BCSA and CHROMagar™ B.cepacia.