Comparison of four commercially available chromogenic media to identify *Candida albicans* and other medically relevant *Candida* species

Ulrike Scharmann\(^1\) | Lisa Kirchhoff\(^1\) | Valerie le Saout Chapot\(^1\) | Jan Dziobaka\(^1\) | Hedda Luise Verhasselt\(^1\) | Raphael Stauf\(^2\) | Jan Buer\(^1\) | Joerg Steinmann\(^1,2\) | Peter-Michael Rath\(^1\)

\(^1\)Institute of Medical Microbiology, University Hospital Essen, Essen, Germany
\(^2\)Institute of Clinical Hygiene, Medical Microbiology and Infectiology, Paracelsus Medical University, Nuremberg, Germany

**Summary**

**Background:** The number of invasive *Candida* infections has significantly increased in recent decades. For the successful treatment of fungal infections, rapid identification at the species level, particularly in polyfungal infections, is a key factor. In this study, four commercially available chromogenic media, CandiSelect™ 4 (CS4), chromID™ Candida Agar (CCA), BBL™ CHROMagar™ Candida Medium (BBL) and Brilliance™ Candida Agar (BCA) were evaluated for *Candida* identification.

**Material/Methods:** Overall, 181 bronchial secretion samples from intensive care patients were analysed prospectively. In addition, 18 primarily sterile materials, previously tested positive for *Candida*, were investigated retrospectively. All samples were cultured as recommended by the manufacturer and visually inspected after 24 and 48 hours by three independent investigators. As a control, colonies were identified by MALDI-TOF MS. Specificity and sensitivity were determined for *C. albicans* identification prospectively.

**Results:** CS4 and BCA showed the best overall consensus with the identification results reached by MALDI-TOF MS for *C. albicans* and species. A clear differentiation between the species could be ascertained via easily identifiable, species-specific coloration in contrast to BBL and CCA. Sensitivity for *C. albicans* (n = 73) identification varied between 32% (BCA) and 69% (CS4 and CCA) after 24 hours and 68% (BBL) and 82% (BCA) after 48 hours incubation, while specificity ranged between 62% (BBL) and 81% (CCA) after 24 hours and 82% (BBL) and 85% (CS4) after 48 hours.

**Conclusion:** CS4 and BCA are recommended for routine identification of *Candida* species in human samples.

**Keywords**

*Candida*, *Candida albicans*, *Candida* species, chromogenic media, culture-based identification, mycological diagnostic, non-*Candida albicans*, yeast identification
1 | INTRODUCTION

The number of invasive infections due to *Candida* spp. has increased in recent years, including an increase in the percentage of infections caused by non-*C. albicans* *Candida* species (NCAC).\(^1\)\(^-\)\(^3\) The shift of species from mainly *C. albicans* towards NCAC in invasive *Candida* infections may be due to the increased application of accurate diagnostic methods and/or an increased in the use of azole therapy.\(^4\)

Thus, the overall *Candida* species shift from antifungal susceptible *C. albicans* to potentially more resistant NCAC isolates, makes the rapid identification of *Candida* isolates at the species level essential.\(^4\)

Routine diagnostic methods for *Candida* identification are commonly culture-based, in which micro- and macromorphology are analysed. Identification through physical characteristics is frequently completed with an array of biochemical tests as part of standard procedures for identifying yeast.\(^5\)

For proper detection of yeast in polymicrobial samples, selective media are crucial, for example Sabouraud dextrose agar (SDA) or malt extract agar. Nevertheless, neither the detection of polyfungal infections, nor the differentiation of species is possible by the sole use of these conventional media. The first report of chromogenic agar for identification and differentiation of *Candida* species was in 1994.\(^6\) The commercially available chromogenic media for *Candida* identification are hexosaminidase-based, enabling the identification of *C. albicans*. However, further discrimination between NCAC species differs in the various media by the use of numerous diverse substrates or by detection of either hexosaminidase activity or alkaline phosphatase activity.

Additionally, bacterial growth in each medium is inhibited, for example, by the use of chloramphenicol and/or gentamicin.\(^7\)-\(^10\)

Since the 1990s, a number of different media have become commercially available. In many studies, either only one or two of these media were compared, using mainly reference strains or strains from internal culture collections.\(^6\)\(^,\)\(^11\)-\(^22\) The latter is not representative for routine diagnostic purposes.

In this study, four different commercially available chromogenic media were compared for their performance in *C. albicans* identification in clinical specimens, including primary sterile materials and respiratory samples. Additionally, performance of the media for NCAC identification was also evaluated.

2 | MATERIALS AND METHODS

2.1 | Media

Four commercially available chromogenic media, CandiSelect™ 4 (CS4, Bio-Rad, Marnes-la-Coquette, France), chromID™ *Candida* Agar (CCA, bioMérieux, Marcy-l’Étoile, France), BBL™ CHROMagar™ *Candida* Medium (BBL, Beckton Dickinson GmbH, Heidelberg, Germany) and Brilliance™ *Candida* Agar (BCA, Oxoid, Basingstoke, UK), were investigated. Each included medium causes a specific characteristic pigmentation as listed in Table 1. In a few cases, colonies were identified using a combination of colour and other morphological observations.

| Medium | Species | Appearance |
|--------|---------|------------|
| CS4    | *C. albicans* | Pink-purple |
|        | *C. tropicalis* | Intensive turquoise, spherical colonies with smooth morphotype |
|        | *C. glabrata* | Pale turquoise/ turquoise colonies with darker centre, flat and shiny with smooth morphotype |
|        | *C. krusei* | Turquoise-blue with characteristically rough morpho-type, a dry appearance and an irregular outline |
| CCA    | *C. albicans* | Blue to dark blue |
|        | *C. tropicalis*, *C. kefyr*, *C. lusitaniae* | Pink |
|        | *C. krusei* | White, characteristic morphology |
| BBL    | *C. albicans* | Light to medium green |
|        | *C. tropicalis* | Dark to metallic blue |
|        | *C. krusei* | Light rose with a whitish border |
| BCA    | *C. albicans*, *C. dublensis* | Green |
|        | *C. tropicalis* | Blue |
|        | *C. krusei* | Brown or pink, dry, irregular colony shape |
|        | *C. glabrata*, *C. kefyr*, *C. parapsilosis*, *C. lusitaniae* | Variable, natural pigment |

Abbreviations: BBL, BBL™ CHROMagar™ *Candida* Medium; BCA, Brilliance™ *Candida* Agar; CCA, chromID™ *Candida* Agar; CS4, CandiSelect™ 4.
FIGURE 1  Morphology after 48 h incubation of *Candida albicans* (A), *Candida dubliniensis* (B), *Candida krusei* (C), *Candida tropicalis* (D) and *Candida glabrata* (E) on: (1) CandiSelect™ 4, (2) chromID™ Candida Agar, (3) BBL™ CHROMagar™ Candida Medium and (4) Brilliance™ Candida Agar
2.2 | Strains and specimens

In the first part of the study, a total of 13 control strains were used for evaluation of growth, colour and texture on chromogenic media: *C. albicans* (DSM 11225, DSM 1386, DSM 11943), *C. dubliniensis* (Reference Stock 2291), *C. krusei* (DSM 70075), *C. tropicalis* (DSM 1346), *C. glabrata*, (DSM 76014), *C. parapsilosis* (DSM 5784), *C. kefyr* (DSM 11954), *C. guilliermondii* (DSM 11947), *S. cerevisiae* (DSM 1333), *E. coli* (ATCC 8739) and *Saureus* (ATCC 25923). Incubation time was 24–72 hours.

In addition to the above listed reference strains, 18 primarily sterile materials (blood cultures, bile, tissues, intraoperative smears) that had tested positive for *Candida* of which 12 were polyfungal in nature (n = 12) as detected in the routine microbiological diagnostic, were investigated retrospectively by testing the four different chromogenic media.

Further, 181 bronchial secretion samples (typically showing a high prevalence of *Candida*) from 181 different patients were plated prospectively onto the four chromogenic media.

2.3 | Cultivation and identification

Incubation temperature was 36°C (CS4, CCA, BBL) or 30°C (BCA) respectively in ambient air as recommended in the manufacturer’s instructions. Plates were assessed after 24 and 48 hours by three independent investigators regarding identification of the yeast and differentiation of polyfungal cultures. Interpretations were performed according to the recommendations of the respective manufacturers.

The reference procedure for identifying yeast species was carried out after cultivation using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS, Vitek MS; bioMérieux, Marcy l’Etoile, France). For identification purposes, each individual colony presenting chromogenic growth was investigated via MALDI-TOF MS. All specimens were cultured additionally on Sabouraud dextrose agar (SDA; Oxoid, Wesel, Germany) and blood agar (Oxoid, Wesel, Germany) at 37°C for 24 and 48 hours, serving as growth control.

2.4 | Data analysis

Statistical analysis was performed to determine sensitivity and specificity for the identification of *C. albicans*, for the prospective part of the study. Due to three independent investigators and the resulting variations in reading, results were depicted as the mode value.

Samples were assigned a true positive result only after microscopic identification was confirmed by MALDI-TOF MS. A false positive result was assigned to cultures incorrectly identified macroscopically, due to a deviation in colony colour from that described by the manufacturer, when compared to MALDI-TOF MS. A false negative reading was assigned if no growth on the medium could be detected but culturing on alternate media was successful. True negative status was assigned in the absence of growth on chromogenic media and on blood agar or SDA.

2.5 | Ethics

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. All clinical samples were used after performing a conventional microbiological analysis. The study did not include patients’ details and did not result in additional constraints for the patients. All procedures and methods were carried out in accordance with approved guidelines.

3 | RESULTS

MALDI-TOF MS analysis results were compared with culture-based identification of *Candida* spp., using the four included chromogenic media. The modal observation of the obtained results from three independent investigators was further used in this study.

In the first instance, the four tested chromogenic media were examined based on their capacity to induce growth. All reference strains grew on each of the tested chromogenic medium with visible colonies after 24 hours. The media differ in their complexity. Figure 1 shows the variety in morphology and pigmentation of colonies after 48 hours incubation of the most common *Candida* species. All included *C. albicans* strains were correctly identified. Furthermore, presumptive identification of *C. krusei* and *C. tropicalis* was possible working within the guidelines set out by the manufacturer. Other species than those mentioned above were not reliably distinguishable. There was no difference in the pigmentation or texture of the colonies after 48 and 72 hours incubation (Figure 2).

In 18 primarily sterile materials, 11 *C. albicans*, 10 *C. glabrata*, 3 *C. tropicalis*, 3 *C. krusei*, 3 *C. lusitaniae*, 1 *C. kefyr*, 1 *C. orthopsilosis* and 1 *C. dubliniensis* were identified by MALDI-TOF MS. Additionally, the analysis of 181 bronchial secretion samples by MALDI-TOF MS revealed 73 *C. albicans*, 32 *C. glabrata*, 9 *C. tropicalis*, 2 *C. krusei*, 2 *C. parapsilosis*, 11 *C. dubliniensis*, 2 *C. kefyr*, 1 *C. inconspicua* and 1 *S. cerevisiae*. On each of the chromogenic media, no bacterial growth was observed from any of the included respiratory samples, which are known to commonly be polymicrobial in nature due to normal respiratory tract flora. Bacterial contamination and overgrowth were thus inhibited by all four chromogenic media.

Comparisons of the results obtained by visual reading after 24 and 48 hours and from MALDI-TOF MS are summarised in Tables 2 and 3. Identification by pigmentation was possible for *C. albicans*, *C. krusei* and *C. tropicalis*. *C. albicans* appeared as pink colonies on CS4, blue on CCA and green on BBL and BCA. *C. krusei* could be identified only through a combination of morphology and pigmentation analysis, due to the characteristic appearance of colonies, with pigmentation serving only as an initial semi-specific marker.
C. tropicalis exhibited a turquoise colour on CS4, pink on CCA and blue on BBL and BCA. Other NCAC were not reliably distinguishable.

A total of 44 samples comprised of 12 primary sterile materials and 32 BAL samples, produced polyfungal profiles when analysed using MALDI-TOF MS. In contrast, the chromogenic agar reading

**TABLE 2** Results of identification of *Candida* on four chromogenic media after 24 and 48 h incubation of 18 primarily sterile materials

| Identity     | Reference | Visual identification |
|--------------|-----------|-----------------------|
|              | MALDI-TOF MS (n) | CS4 24 h (n (%)) | CCA 24 h (n (%)) | BBL 24 h (n (%)) | BCA 24 h (n (%)) | CS4 48 h (n (%)) | CCA 48 h (n (%)) | BBL 48 h (n (%)) | BCA 48 h (n (%)) |
| *C. albicans* | 11        | 9 (82)               | 8 (73)            | 4 (36)            | 4 (36)            | 12 (109)         | 11 (100)          | 10 (91)           | 11 (100)          |
| *C. dubliniensis* | 1 | 0 (0)               | 0 (0)             | 0 (0)             | 0 (0)             | 0 (0)            | 0 (0)             | 0 (0)             | 0 (0)             |
| *C. krusei*    | 3         | 2 (67)               | 3 (100)           | 4 (133)           | 2 (67)           | 3 (100)          | 2 (67)            | 3 (100)           | 3 (100)           |
| *C. tropicalis* | 3       | 1 (33)               | 1 (33)            | 3 (100)           | 2 (67)           | 3 (100)          | 3 (100)           | 3 (100)           | 3 (100)           |
| *C. glabrata*  | 10        | 1 (10)               | 0 (0)             | 0 (0)             | 1 (10)           | 1 (10)           | 1 (10)            | 0 (0)             | 5 (50)            |
| *Candida spp.* | 5         | 0 (0)               | 0 (0)             | 0 (0)             | 0 (0)            | 0 (0)            | 0 (0)             | 0 (0)             | 0 (0)             |
| Polyfungal Sample | 12 | 7 (58)               | 8 (67)           | 5 (42)            | 4 (33)           | 11 (92)          | 9 (75)            | 15 (125)          | 10 (83)           |

Abbreviations: %, percent; BBL, BBL™ CHROMagar™ Candida Medium; BCA, Brilliance™ Candida Agar; CCA, chromID™ Candida Agar; CS4, CandiSelect™ 4; h, hours; n, Number of isolates.
revealed polyfungal cultures in numbers between 12 and 22, when read after 24 hours, or 32 and 35, when read after 48 hours respectively with higher detection rates for primary sterile material (Table S1). However, a comparison of respiratory and primarily sterile materials showed no discernable differences in colour or morphology of the colonies between samples of different origins on the agar. The overall highest detection rates for polyfungal samples have been observed using CS4 (n = 34, 77%) and BCA (n = 35, 80%) after 48 hours. In contrast, CCA (n = 32, 73%) and BBL (n = 33, 75%) showed poorer results for detection of polyfungal samples. While the identification of \textit{C. albicans} from polyfungal samples has been shown to be more accurate, identification of other \textit{Candida} species has been shown to be problematic, resulting more often in no possible identification or misidentification. The most frequent error was in identifying \textit{C. glabrata} incorrectly identified as \textit{C. tropicalis} and \textit{C. dubliensis} being misidentified as \textit{C. albicans}. Overall, the most efficacious reading of results from each of the included media was reached when visual analysis was performed after 48 hours as opposed to after 24 hours.

Species-specific sensitivity and specificity were calculated for \textit{C. albicans} (Table 4). Sensitivity varied between 32% (BCA) and 69% (CS4 and CCA) after 24 hours and 68% (BBL) and 82% (BCA) after 48 hours, while specificity ranged between 62% (BBL) and 81% (CCA) after 24 hours and 82% (BBL) and 85% (CS4) after 48 hours. Best specificity (85% and 83%) and sensitivity (81% and 82%) was exhibited by CS4 and BCA after 48 hours. Because of the low number of identified NCAC, no sensitivity and specificity were defined for NCAC identification.

### 4 | DISCUSSION

Due to the increasing numbers of \textit{Candida} positive clinical specimens, the need for rapid and accurate species identification is of great importance. For this purpose, the use of standard fungi media as SDA provides insufficient information, allowing only the observation of positive or negative yeast growth without reliable identification of polyfungal cultures. Therefore, chromogenic media are frequently applied to detect and identify yeast of the \textit{Candida} genus at the species level in a more rapid and cost-efficient way. There are various studies comparing the performance of chromogenic media to identify \textit{Candida} species and to detect polyfungal infections.\textsuperscript{6,11-22} To our knowledge, this is the first study evaluating and comparing the performance of the currently commercially available CS4, CCA, BBL and BCA for accurate and rapid identification and differentiation of \textit{Candida} species from clinical specimens.

Identification of \textit{C. albicans} was reliable using each of the included media as previously reported.\textsuperscript{12,15,19} A study including 1549 specimens with 32% testing positive for yeast found the best evaluation time point to be after 48 hours of incubation when culturing on CS4.\textsuperscript{12} Guzel et al compared the performance of CCA to CHROMagar Candida and SDA by investigating 205 fungus-positive vaginal swab samples. They identified 64% of \textit{C. albicans} isolates after 48 hours.\textsuperscript{17} This identification rate was lower compared with our results. Another study detected high values for sensitivity (100%) and specificity (95.3%) after 48 hours on CCA.\textsuperscript{15} While Ozcan et al demonstrated a high sensitivity (87.5%) and specificity...
TABLE 4 Sensitivity and specificity of chromogenic media for C. albicans after 24 h and 48 h incubation of prospective analysis (N = 73 for C. albicans detection in MALDI-TOF-MS serving as reference)

|                | CS4 (n) | CCA (n) | BBL (n) | BCA (n) |
|----------------|---------|---------|---------|---------|
| Incubation for 24 h |         |         |         |         |
| True positive      | 19 ± 13 | 19 ± 13 | 19 ± 15 |         |
| False positive     | 53 ± 4  | 20 ± 2  | 20 ± 2  | 20 ± 2  |
| False negative     | 28 ± 3  | 28 ± 3  | 28 ± 3  | 28 ± 3  |
| True negative      | 86 ± 0  | 86 ± 0  | 86 ± 0  | 86 ± 0  |
| Sensitivity (%)    | 69      | 69      | 69      | 69      |
| Specificity (%)    | 74      | 81      | 81      | 81      |
| Incubation for 48 h |         |         |         |         |
| True positive      | 56 ± 1  | 56 ± 1  | 68 ± 2  |         |
| False positive     | 17 ± 5  | 19 ± 4  | 19 ± 4  | 17 ± 2  |
| False negative     | 26 ± 0  | 26 ± 0  | 26 ± 0  | 26 ± 0  |
| True negative      | 86 ± 0  | 86 ± 0  | 86 ± 0  | 86 ± 0  |
| Sensitivity (%)    | 81      | 80      | 82      | 82      |
| Specificity (%)    | 85      | 83      | 82      | 83      |

Note: Data were analysed by taking the average of three independent reviewers for each statement, thus varying total numbers are resulting. Averages were used to calculate the sensitivity and specificity on basis of 73 true positive C. albicans isolates. 

(100%) when using BBL to identify C. albicans;11 this study detected C. albicans with only 68% sensitivity and 82% specificity using BBL. Reasons for the disparity in performance of BBL in the aforementioned studies may be due to variations in the types of samples tested.12 Baxien et al compared BCA and CHROMagar Candida and determined a sensitivity of 96.6% and a specificity of 100% for identifying C. albicans after 48 hours.19 In contrast, after 24 hours incubation, only 19 of 89 colonies showed a characteristic colour,19 which was also observed in the current study. Here, CS4 and BCA showed the most robust positive results and the highest sensitivity for C. albicans identification, whereas the highest specificity was obtained using CS4, CCA and BCA, when identifying colonies macroscopically after 48 hours of incubation.

We were able to identify C. krusei on all included agar media. This was only possible when morphological characteristics of the colonies were analysed in concert with pigmentation assessment. Similar observations have been reported in other studies.11-14,17,18 We could observe that, on BBL agar, non-krusei Candida species are almost indiscernible from C. krusei after the first 24 hours. Each of the false positive C. krusei isolates was identified as C. glabrata. Additionally, nearly all of these C. glabrata isolates have been shown to originate from polymicrobial samples also containing C. albicans. Only one misidentified C. krusei has been confirmed to be a pure culture of C. glabrata. However, those false positive results did not occur when identification was performed after 48 hours of incubation.

A relatively low identification rate was reached for C. tropicalis on the chromogenic media with the exception of BBL, which enabled the true positive identification of 83% of C. tropicalis. On BBL, C. tropicalis develops a unique and easy-to-identify colour. This has similarly been observed for BCA with a 75% identification rate. Vecchione et al were able to detect C. tropicalis properly after 24 hours on CS4, BBL and BCA, but this study only included yeast isolates from a strain collection.14 Another study group reported 95% sensitivity and 79% specificity when 21 C. tropicalis isolates were investigated on CCA.15 Ozcan et al were not able to detect differences in colony colour between C. krusei and C. tropicalis, at any time point of observation on BBL or BCA.11

Identification of C. glabrata was not possible using any of the chromogenic media and C. dubliniensis could only be detected in a few cases (n = 4, 33%) on BCA agar. Both species have been observed to be similar to NCAC and to C. albicans in pigmentation respectively when cultured on chromogenic media.11,14,16-18,21,22 Vecchione et al reported that they were able to differentiate between C. albicans and C. dubliniensis on CCA, BBL and BCA.14 C. dubliniensis is not listed in the literature accompanying the media as a detectable species.7-10

For the identification of polyfungal cultures, an incubation time of 48 hours produced the most robust results in our study. In the literature, the identification of polyfungal cultures was elucidated after 72 hours with reliable results for all chromogenic media, except BBL.11-12,17 Ozcan et al detected only 66.7% (14 of 21) of polyfungal cultures after 72 hours incubation.11

A limitation of our study might be the inclusion of only low numbers of samples. In addition, the evidence of yeast species identification in respiratory samples is controversial. It is known, that yeast are a rare cause of pneumonia.24 Further study is required to evaluate chromogenic media for the identification of yeast from relevant clinical specimens.

Summing up, all chromogenic media allowed the culture of Candida species from clinical specimens. In general, the most specific morphologies of the detected species were exhibited after 48 hours of incubation. Candida identification on the species level relied mainly on such morphological aspects as colour as well as texture of colonies. The commercially available chromogenic media differ in their suitability for Candida identification on the basis of varying colony pigmentation mechanisms. In this study, CS4 and BCA agar were defined as the most suitable chromogenic media for identification of C. albicans. In contrast to C. albicans, identification of NCAC at the species level and distinguishing between NCAC is more difficult as various NCAC exhibit the same or similar colony pigmentation.14,15,21,23 It is therefore recommended, that an identification of NCAC should be performed using more reliable and rapid
methods, for example, MALDI-TOF MS, enabling an identification on species level for sufficient microbiological diagnosis and consequently appropriate therapy.

In conclusion, most promising results for the detection of Candida spp. in polyfungal cultures were exhibited by CS4 after 24 hours and BCA after 48 hours incubation with clearest and most distinguishable reading results and highest sensitivity and specificity when compared to CCA and BBL. Thus, CS4 or BCA is recommended for routine laboratory use for the selective isolation and identification of yeast from clinical specimens.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

AUTHOR CONTRIBUTION

Ulrike Scharmann: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Methodology (lead); Project administration (equal); Software (equal); Supervision (lead); Validation (lead); Visualization (equal); Writing-original draft (equal); Writing-review & editing (equal). Lisa Kirchhoff: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Project administration (equal); Software (equal); Supervision (equal); Writing-original draft (equal); Writing-review & editing (equal). Valerie le Saout Chapot: Data curation (equal); Methodology (equal). Jan Dziobaka: Data curation (equal); Methodology (equal). Hedda Luise Verhasselt: Data curation (equal); Investigation (equal); Methodology (equal). Raphael Stauf: Data curation (equal). Jan Buer: Project administration (equal); Supervision (equal). Joerg Steinmann: Conceptualization (equal); Writing-original draft (supporting); Writing-review & editing (supporting). Peter-Michael Rath: Conceptualization (supporting); Data curation (supporting); Project administration (supporting); Writing-original draft (supporting); Writing-review & editing (supporting).

AUTHOR CONTRIBUTIONS

US, P.-MR conceived the experimental design; US, VC, JD, HLV, RS collected the data; US, JK, JS, P.-MR analysed the data; US, JK, JB, JS, P.-MR critical reading and discussion of results; and US led the writing.

ORCID

Ulrike Scharmann https://orcid.org/0000-0001-7689-7799

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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