Evaluation of selective media for the isolation of *Burkholderia cepacia* complex from respiratory samples of Cystic Fibrosis patients

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INTRODUCTION

Cystic Fibrosis (CF) is a life limiting inherited disease caused by a mutation in the CF transmembrane conductance regulator (CFTR) gene. This causes reduced mucociliary clearance of the lungs, allowing bacteria that are normally removed from the respiratory tract to multiply, resulting in colonisation and infection. Colonisation with species of the *Burkholderia cepacia* complex (Bcc) are associated with a significant increase in morbidity and mortality and can be a contraindication to lung transplantation. Accurate identification of Bcc from CF patients is vital because of the impact that diagnosis can have on patient management.

METHOD

The aim of this study is to evaluate the performance of Bcc selective media modified with gentamicin (MOD; E&O Laboratories Ltd), and Colorex *B. cepacia* media (Colorex; Chromagar), using Bcc selective media (CEP; E&O Laboratories Ltd) as the comparator. The media was challenged with a range of isolates taken from an internal collection, clinical samples, spiked samples, and control organisms.

Clinical samples: 141 clinical respiratory samples including cough swabs (n=85), sputum (n=46), sinus aspirate (n=6) and saliva samples (n=4) were taken from patients with CF. Samples were processed according to the local SOP and inoculated onto all three media. All media was incubated aerobically at 37°C and examined at day 1, day 2 and day 7. All growth was recorded and identified using MALDI-Tof.

Spiked samples: Samples known to be negative for Bcc were homogenised and divided into a panel of 20 samples that were 'spiked' with Bcc and non-Bcc species from an internal collection.

Limit of Detection (LOD): *B. cepacia* (NCTC 10661) was diluted in logarithmic dilutions into saline and inoculated into sputum to create samples with a series of concentrations ranging from 10^1 to 10^5 colony forming units (CFU)/mL. Performed in triplicate.

Selectivity: Each media was challenged with 10 non-Bcc organisms.

RESULTS

Out of 161 clinical and spiked samples, Bcc was isolated on CEP media from 20 samples, and on MOD and Colorex media from 19 samples after a 7-day incubation period. All 'spiked' samples were concordant across all three media.

Both media had a sensitivity, specificity, PPV, and NPV of 95%, 100%, 100%, and 99.3% respectively when compared to the CEP medium. Non-Bcc species were isolated from 9.9% of respiratory samples inoculated on CEP media (n=16), 7.5% on MOD media (n=12), and 1.9% on Colorex media (n=3).

Performance Parameter	Value (95% CI) MOD and Colorex
Sensitivity	95.2% (76.9% - 99.9%)
Specificity	100% (97.4% - 100%)
Positive Predictive Value (PPV)	100% (83.2% - 100%)
Negative Predictive Value (NPV)	99.3% (95.4% - 99.9%)
Accuracy	99.4% (96.6% - 99.9%)

Table 1. Performance parameters for both MOD and Colorex media when compared to CEP media.

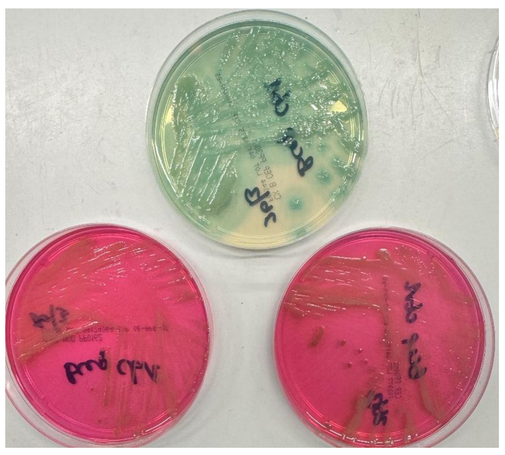

Figure 1. *B. cepacia* (NCTC 10661) appearance on Colorex (top), MOD (bottom left), and CEP (bottom right)

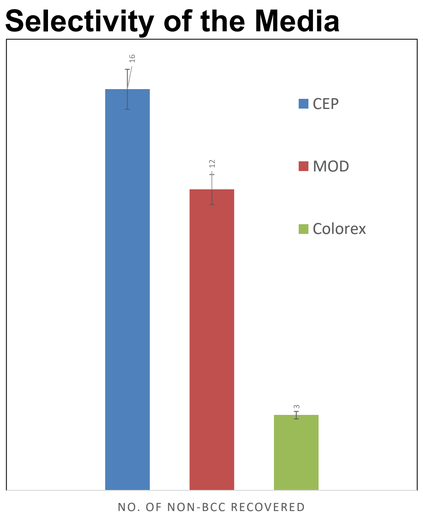

Figure 2. Chart comparing the selectivity of the media

RESULTS (continued)

After 7 days incubation Bcc was reproducibly isolated on CEP and MOD media at 10^3 CFU/mL and on Colorex media at 10^2 CFU/mL.

9 out of 10 non-Bcc bacterial isolates were inhibited by all three media.

Concentration	CEP Day 1	CEP Day 7	MOD Day 1	MOD Day 7	Colorex Day 1	Colorex Day 7
10^1 CFU/mL	0/3	0/3	0/3	0/3	0/3	0/3
10^2 CFU/mL	0/3	1/3	0/3	1/3	0/3	3/3
10^3 CFU/mL	0/3	3/3	0/3	3/3	3/3	3/3
10^4 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3
10^5 CFU/mL	3/3	3/3	3/3	3/3	3/3	3/3

Table 2. Limit of detection performed in triplicate.

Control organism	Growth on Day 7		
	CEP	MOD	Colorex
S. maltophilia	No Growth	No Growth	No Growth
A. xylosoxidans	No Growth	No Growth	No Growth
A. baumannii	No Growth	No Growth	No Growth
E. faecalis	No Growth	No Growth	No Growth
K. pneumoniae	No Growth	No Growth	No Growth
E. coli	No Growth	No Growth	No Growth
P. mirabilis	Growth (Pink halo)	Growth (Pink halo)	Growth (Non-pigmented colonies)
P. aeruginosa	No Growth	No Growth	No Growth
S. aureus	No Growth	No Growth	No Growth
C. albicans	No Growth	No Growth	No Growth

Table 3. Control organisms used to challenge each media and the results from Day 7.

DISCUSSION

From 161 clinical and spiked respiratory samples, 20 Bcc were isolated on CEP media, and 19 Bcc were isolated on MOD and Colorex media. One clinical sample failed to grow Bcc on both the MOD and Colorex resulting in a 95.2% clinical sensitivity for both media. For this sample only 2 colonies were isolated on the CEP medium after 7 days of incubation, indicating a low bacterial load that was close to the limit of detection for the media where results cannot be reliably reproduced.

In this study, the LOD analysis showed that Colorex media had a higher analytical sensitivity, as Bcc was reproducibly isolated at a lower concentration after 1- and 7-days incubation than CEP and MOD media.

Both MOD and Colorex media displayed higher selectivity for Bcc than CEP media, with the Colorex media being particularly effective at inhibiting non-Bcc species. All three media performed well to inhibit non-Bcc control organisms. The only outlier was *Proteus mirabilis,* which was able to grow on all three media, with a non-typical appearance expected for Bcc colonies on Colorex.

Specificity, PPV, NPV, and accuracy were 100%, 100%, 99.3%, and 99.4% respectively, providing reassurance that both MOD and Colorex are reliable media for the isolation of Bcc. The characteristic blue pigmentation of Bcc colonies on Colorex media was often visible after 1-day of incubation and was a useful aid in the presumptive identification of Bcc.

A limitation of this study is the low number of Bcc positive clinical samples; a larger study to capture more Bcc positive patients would be beneficial to further assess the performance of the media.

To conclude, all three media achieved high sensitivity, specificity, PPV, and NPV for the detection of Bcc from respiratory samples from patients with CF. Colorex media displayed higher selectivity and allowed for earlier detection and easier interpretation of Bcc isolates which could be beneficial when used in the clinical laboratory.

REFERENCES

Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis Second edition. (2022). *Cystic Fibrosis Trust*.

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Thank you to E&O Laboratories Ltd. and Chromagar for supplying the Bcc selective media modified with gentamicin (MOD) and Colorex *B. cepacia* media free of charge.

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