Evaluation Study on Antimicrobial Neutralization with Automated Blood Culture Systems at Sunway Medical Centre

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ABSTRACT

This study compared the BioMeriéux BacT/ALERT® blood culture (BC) system with Becton Dickinson BACTEC 9120 BC system for recovery and time to detection (TTD) of common isolates from Sunway Medical Centre (SunMed) in the presence of antimicrobial drugs in culture media. A total of 108 bottles were included in the study, which was carried out for a duration of two months from September till November 2014 . 54 bottles were incubated at each BC system. In addition to that, a total of 108 bottles without adding antimicrobial drugs were also tested as controls. The results demonstrated that no isolates were detected for *Staphylococcus aureus* (SA) and Methicillin-resistant *Staphylococcus aureus* (MRSA) with presence of Vancomycin for both systems. A substantial difference was noted between BacT/ALERT® and BACTEC for recovery of seeded bacteria. Gram-Negative Bacilli (GNB) is most recovered bacteria by both the systems as compared to Gram-Positive Cocci (GPC). Further more, the mean TTD of seeded bacteria with antimicrobial presence was substantially less with BacT/ALERT® as compare to BACTEC.

BACKGROUND

Excellent Quality Management practice as set by the local requirement standard ; MS ISO 15189 requires evaluation of new method prior to introducing it in the existing system. Therefore, to compile with the standards, we had done an evaluation study on current BC system i.e. BACTEC with the new BC system from BioMeriéux i.e. BacT/ALERT®. BACTEC system uses fluorescent method to detect bacteria growth in the BC bottle whereas BacT/ALERT® uses colorimetric method.

OBJECTIVE

This study compared the ability of BacT/ALERT® Plus BC media and BACTEC™ Plus™ BC media to neutralize antimicrobial drugs such as Cefuroxime (CXM), Piperacillin-tazobactam (TZP) and Vancomycin (Va). The microorganism used in this challenge are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeroginosa* and MRSA. The selection of the antimicrobial and bacteria were based on common usage of the antimicrobial drugs and bacteria isolates at SunMed.

MATERIALS AND METHOD

A) Study overview

This is a comparative evaluation study of BacT/ALERT® Plus media and BACTEC Plus media and their ability to remove various antimicrobial drug from BC specimens (antimicrobial neutralization activity). The selected bacteria will be seeded in donor's whole blood that is less than 5 days old which is kept and stored at 2 – 8 °C. Combination of bacteria and antimicrobial will form 5 cycles and the 6th cycle is the experiment to study on less blood volume usage in blood culture bottle.

Test bottles were inoculated in triplicate for all cycles. Once BC bottle are inoculated with bacterium and antimicrobial suspension, they will be placed immediately in their respective instruments to prevent delayed vial entry effects that may exist for either systems (Refer **Flow Chart 1**).

Cycle	1	2	3	4	5	6
Combination	*E.coli* with CXM	*E.coli* with TZP	*P. aeruginosa* with TZP	*S.aureus* with VA	MRSA with VA	*E.coli* with CXM (less blood volume)

(B) Preparation on antimicrobial peak concentration

i. Antimicrobial used for testing: Cefuroxime (CXM), Piperacillin-tazobactam ,Tazocin (TZP) and Vancomycin (Va).
ii. Antimicrobial solution will be prepared from commercial drug powder.
iii. Each antibiotic will be tested at peak concentration only as shown in table below:

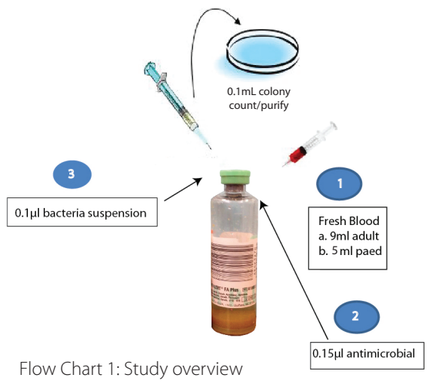
No	Antibiotic (Generic)	Commercial Brand	Concentration	Peak Concentration tested
1	Cefuroxime	Zinacef	750mg/ml	100ug/ml
2	Piperacillin-Tazobactam	Tazocin	45g/20ml	50ug/ml
3	Vancomycin	Vancomycin	500mg/10ml	75ug/ml

(C) Preparation on bacteria suspension

i. Overnight growth for selected bacteria on the appropriate plated culture media.
ii. Perform serial dilution to obtain 10 – 100 colony form unit (CFU) inoculum range with the turbidity at 1.0 McFarland.
iii. To get desired peak concentration, 0.15 µl final dilution added to each BC bottle.

(D) Inoculum of BC bottle with antimicrobial and bacteria suspension

i. BC bottles used for testing:
  i. BacT/ALERT®: FA Plus (Aerobic); FN Plus (Anaerobic) and PF Plus (Aerobic Paediatric).
  ii. BACTEC™ Plus™ : Aerobic/F; Anaerobic/F and Paeds/F.
ii. Inject fresh donor's blood into each BC bottle(Adult: 9mL; Paeds: 5 mL). Invert the BC bottle to mix.
iii. Inject 0.15µL of final constituted antimicrobial drug into each BC bottle.
iv. Inoculate all BC vial in triplicate with 0.1µL of the organisms.
v. Invert the BC bottle to mix and enter them in the appropriate automated BC instruments immediately.
vi. All positive bottles were subculture to ensure purity.



Flow Chart 1: Study overview

EXPECTED RESULTS:

1. Consider growth detection with 5 days as successful antimicrobial neutralization.
2. Bottles in which no growth is detected after 5 days will be reported as Negative for growth.
3. Bottles in which growth is detected will be plated on blood agar media to check on purity and bacteria inoculum count.

RESULTS

The results demonstrated that no isolates detected for *Staphylococcus aureus* (SA) and Methicillin-resistant *Staphylococcus aureus* (MRSA) with present of Vancomycin for both systems.
Overall TTD for control bottle is shown in **Table 1** where there was no growth detected for *Pseudomonas aeruginosa* for both system.
Overall mean TTD for tested seeded bacteria shown in **Table 2**. For individual mean TTD results for each cycle shown at **Graph 1** to **Graph 5**.
The mean of TTD of seeded bacteria with antimicrobial was substantially less with BacT/ALERT® then BACTEC system. TTD with less blood volume shown in **Graph 6**.

For recovery rate by type of blood culture bottle, 18/18 (100%) were from BacT/ALERT®FA Plus (Aerobic) bottles and 11/18(61%) were from BACTEC™ Plus Aerobic/F; 9/15(60%) were from BacT/ALERT®FN Plus (Anaerobic) bottles and 5/15(33%) were from BACTEC™ Plus Anaerobic/F bottle; 17/18(94%) were from BacT/ALERT®PF Plus (Aerobic Paediatric) and 18/18(100%) were from BACTEC™ Paeds Plus™/F [**Table 3**].
Total of recovered vial on BC bottle by bottle type for both system shown in **Graph 7** while total for recovered vial by system shown in **Graph 8,** BacT/ALERT® system yielded 44/51 (86%) and BD BACTEC™ 9120 system yielded 34/51 (67%).

Time to Detection, h - Positive Control Bottle							
	Cycle #	1	2	3	4	5	6
	Drug	Cefuroxime	Pip/Tazo	Pip/Tazo	Vancomycin	Vancomycin	Cefuroxime
	Organism	*E.coli*	*Ps.aeruginosa*	*E.coli*	*S.aureus*	MRSA	*E.coli (less blood vol)*
Aerobic	**BacT/Alert**	10.08	16.80	11.52	12.96	13.68	12.24
	BACTEC	9.71	18.88	13.54	12.42	13.21	12.54
Anaerobic	**BacT/Alert**	9.12	**No growth**	10.80	13.92	13.44	11.28
	BACTEC	10.21	**No growth**	10.87	12.92	13.37	14.70
Aerobic Pediatric	**BacT/Alert**	9.60	16.56	11.04	13.68	12.72	11.76
	BACTEC	9.21	16.04	10.37	15.25	15.05	11.20

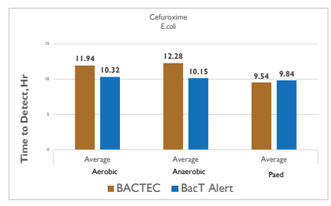
Table 1: Time to Detection for Positive Control Bottle

Time To Detection(TTD), hour - Average							
	Cycle #	**1**	**2**	**3**	**4**	**5**	**6**
	Drug	Cefuroxime	Pip/Tazo	Pip/Tazo	Vancomycin	Vancomycin	Cefuroxime
	Organism	*E.coli*	*Ps.aeruginosa*	*E.coli*	*S.aureus*	MRSA	*E.coli (less blood vol)*
Aerobic	**BacT/Alert**	10.32	17.28	11.60	23.36	24.24	12.56
	BACTEC	11.94	22.47 (1)*	19.06 (2)*	45.41 (2)*	23.73 (2)*	14.54 (1)*
Anaerobic	**BacT/Alert**	10.15	No growth	11.36	No growth	No growth	11.52
	BACTEC	12.28	No growth	16.04 (1)*	No growth	No growth	13.70 (1)*
Aerobic Pediatric	**BacT/Alert**	9.84	18.48	11.44	14.00	14.64	12.12 (2)*
	BACTEC	9.54	18.74	12.09	19.44	20.86	13.31

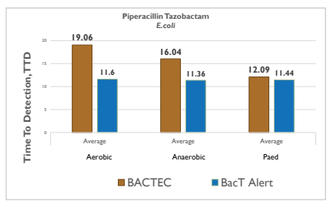
Table 2: Time to Detection for Bottles tested (Average)

Recovery of Pathogen from Blood Culture Bottles With Addition of Antibiotic						
	BACTEC			**BacT/Alert**		
	No. Tested	No. Positive	*% Positive*	No. Tested	No. Positive	*% Positive*
Aerobic	18	11	*61%*	18	18	*100%*
Anaerobic	15	5	*33%*	15	9	*60%*
Aerobic Pediatric	18	18	*100%*	18	17	*94%*
Total	**51**	**34**	***67%***	**51**	**44**	***86%***

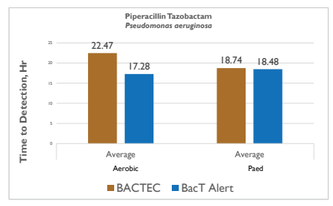
Table 3: Percentage (%) Positive for bottle tested



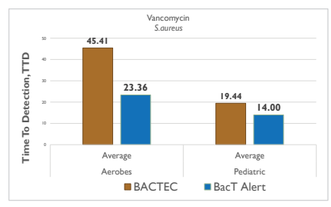
Graph 1: *E.coli* with CXM



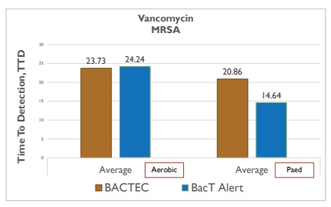
Graph 2: *E. coli* with TZP



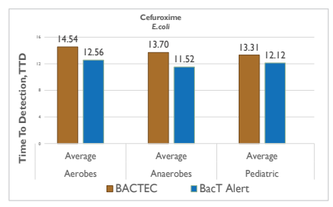
Graph 3. *Ps. aeruginose* with TZP



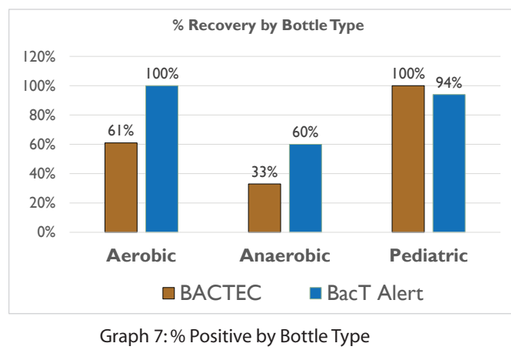
Graph 4: *S.aureus* with VA



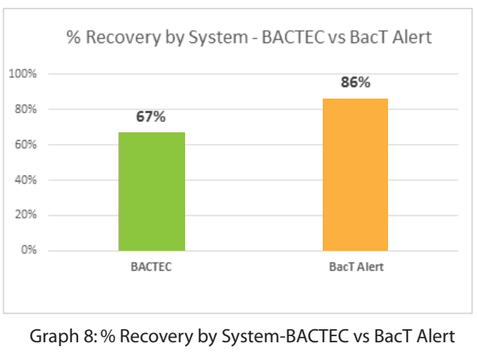
Graph 5: MRSA with VA



Graph 6: *E.coli* with CXM(Less blood volume)



Graph 7: % Positive by Bottle Type



Graph 8: % Recovery by System-BACTEC vs BacT Alert

Discussion and Limitation

The efficiency of the neutralization and absorption of antimicrobial agents by these substances is expected to significantly impact the time to detection (TTD) and recovery of microorganism[1]. Our comparison study demonstrated that mean TTD of seeded bacteria with antimicrobial agent tested was substantially shorter for BacT/ALERT® and total recovery rate substantially more for BacT/ALERT® . The comparison study was done on small sample size due to time and cost restriction. Limitation on apparatus i.e. large and inaccurate scale on syringe and dilution tube; non sterile conical tube for dilution; variances in viability of bacteria suspension after preparation; uncertain on final peak concentration prepared; inconsistency of vial vacuum pressure on each vials will effect on the analysis finding need to be considered.

CONCLUSIONS

The overall detection rate in BacTALERT ® Plus bottles is substantially had a higher detection rate than Bactec Plus bottle (86% and 67% respectively) which indicative of better antimicrobial neutralization in BacTAlert® Plus bottle

References: 1. Comparison of Antimicrobial Binding and Growth Kinetics in BacT/ALERT®FA Plus and BD BACTEC™ Plus Aerobic Media D. Lovern*, B. Katzin, K. Johnson, E. Miller, A. Gates, P. Deol, K. Doing, W. M. Dunne, Jr. bioMérieux Inc., Durham, NC, USA. 2. Comparative Evaluation of BacTALERT® PLUS, Bactec® FX and VersaTREK® Blood Culture Systems with stimulated adult and paediatric bacteremia conditions Beaudoin MC 1,2,3, Gervais P 1,2,3, Ruest A 1, Loungnarath V 1, Longtin Y 4, Bédard C1, Martineau P2, St-Amand C1, Morin K1, Longtin J1,3 1 CHU de Québec, 2 IUCPQ, 3 Centre de Recherche en Infectiologie de l'Université Laval, Québec City, 4 McGill University , Montréal ; (Québec) CANADA. 3. Performance of Two Resin-Containing Blood Culture Media in Detection of Bloodstream Infections and in Direct Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) Broth Assays for Isolate Identification: Clinical Comparison of the BacT/Alert Plus and Bactec Plus Systems .Barbara Fioria, Tiziana D'Inzeoa, Viviana Di Florioa, Flavio De Maioa, Giulia De Angelisa, Alessia Giaquintoa, Lara Campanab, Eloisa Tanzarellac, Mario Tumbarellob, Massimo Antonellic, Maurizio Sanguinettia and Teresa Spanua . J.Clin.Microbiol. 2014, 52(10):3558. DOI:10.1128/JCM.01171-14. 4. Comparison of BacT/ALERT® FA and FN Blood Culture Bottles to BacT/ALERT FA Plus and FN Plus Bottles for Effectiveness of Antibiotic Neutralization and Time to Positivity. Christian M. Hill 1,2, Valerie Hadwell1, Heather Jewsbury1, Edith M. Blondel-Hill 1,3 1 Kelowna General Hospital, Interior Health, British Columbia, 2 University of British Columbia - Okanagan, 3 University of British Columbia – Vancouver 5. Neutralisation of Antimicrobial Subtances in New BacT/Alert FA and FN Plus Blood Culture Bottles Dieter Mitteregger, Wolfgang Baraousch, Marion Nehr, Michael Kundi, Markus Zeitlinger, Athanasios Makristathis and Alexander M.Hirsch J.Clin. Microbiol. 2013, 51(5):1534. DOI:10.1128/JCM.00103-13