

Investigating Rapid Microbial Detection Methods (RMM) as an alternative to Total Viable Counts (TVC) for Non-sterile bioburden assessments



Sarah Hiom[†], Stephen Denyer[‡], Antony Wilkes^{*}, Catherine Talbot[‡]

[†]Cardiff and Vale University Health Board, Cardiff [‡]Welsh School of Pharmacy, Cardiff University

^{*}Anaesthetics, Intensive Care and Pain Medicine, School of Medicine, Cardiff University.

Introduction

RMM has been shown to improve aseptic NHS QA activities¹.

This study investigates the potential to use RMM for non-sterile bioburden testing. The dilution to extinction principle could allow the use of a presence/absence RMM test (BacT/ALERT) to predict original sample bioburden.

To explore this principle further, equivalence of the novel RMM method in question must be established against the gold standard TVC.²

Aims

This study will investigate equivalence between BacT/ALERT and TVC methods at the lower levels of detection and propose how this information may be used to assess microbial quality of a non-sterile product sample.

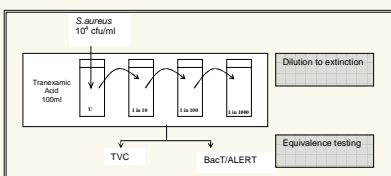
Method

100ml product (Oral Tranexamic acid M/W 5%) was inoculated with *S.aureus* (Bioball bioMerieux) to final concentration of 100cfu/ml. Eight separate samples were then serially diluted to 1 in 1000 using peptone buffer solution (bioMerieux).

Detection of *S.aureus* at each dilution step, through to extinction, was then determined using both the traditional Total Viable Count method² and the RMM - BacT/ALERT. Split sampling methodologies were employed³. See Fig 1.

Equivalence testing for this RMM vs traditional TVC was initially assessed using EN ISO 16140:2003³ (industrial standard for equivalence) and subsequently with non-inferiority statistical analysis.

Fig 1. Method



Results

Table 1. Results from RMM and TVC for each dilution series

Dilution	Repeat	Runs															
		1		2		3		4		5		6		7		8	
		TVC	RMM	TVC	RMM	TVC	RMM	TVC	RMM	TVC	RMM	TVC	RMM	TVC	RMM	TVC	RMM
Undiluted	1	90	N/A	104	N/A	104	N/A	100	N/A	110	N/A	114	N/A	90	N/A	92	N/A
1 in 10	1	11	1.05	6	1.19	10	1.24	8	1.27	7	1.37	9	1.34	12	1.24	5	1.24
	2									2	1.33	0	1.39	3	1.44	2	-
	3									2	1.36	3	1.41	0	1.33	2	1.36
	4									1	1.36	1	1.38	3	1.35	0	1.37
	5									2	1.37	3	1.37	2	1.37	1	1.44
	6									1	1.38	1	1.29	2	1.28	1	1.41
	7									2	1.33	2	1.37	0	1.23	1	1.13
	8									3	1.37	3	1.39	1	-	1	1.39
	9									1	1.32	2	0	1.39	3	1.48	-
	10									1	1.33	1	1.38	0	1.34	2	1.52
1 in 100	1	2	1.30	2	1.40	0	1.46	2	1.48	0	-	1	1.42	0	1.53	0	1.4
	2	1	-	2	1.34	0	1.28	2	-	0	-	0	-	0	-	0	-
	3	0	-	0	-	0	-	0	1.50	2	-	1	-	0	1.3	0	1.48
	4	1	-	2	-	0	1.41	1	1.46	2	1.45	0	1.39	1	-	1	1.49
	5	0	1.23	0	1.39	0	1.24	2	1.36	0	1.35	0	1.46	0	1.51	0	1.46
	6	0	-	2	1.32	0	1.41	0	-	1	1.35	2	1.35	0	1.38	1	1.45
	7	0	1.24	3	1.23	2	-	1	1.37	0	-	0	-	2	-	0	1.43
	8	1	1.08	0	1.27	3	-	2	1.31	1	1.51	0	-	4	1.31	1	1.4
	9	1	1.19	0	1.38	0	1.29	1	1.65	0	-	0	1.39	0	1.42	1	1.32
	10	1	1.23	3	1.32	0	1.40	0	1.48	0	1.29	1	1.42	0	1.27	0	1.32
1 in 1000	1	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
	2	0	-	0	-	1.40	0	-	0	-	0	-	0	-	0	-	0
	3	0	-	0	-	0	-	0	1.49	-	-	-	-	-	-	-	-
	4	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
	5	0	-	0	-	0	1.42	0	-	-	-	-	-	-	-	-	-
	6	0	-	0	-	0	-	0	-	-	-	-	-	-	-	-	-
	7	0	1.22	0	-	0	1.34	0	-	-	-	-	-	-	-	-	-
	8	0	-	0	-	0	-	0	-	-	-	-	-	-	-	-	-
	9	0	-	0	-	0	-	0	-	-	-	-	-	-	-	-	-
	10	0	-	0	-	1	-	0	-	-	-	-	-	-	-	-	-

* Time to detection

° colony forming units

BacT/ALERT®

CO₂ generated by live microbes → detectable colour change



The comparative results between RMM (time to detection) and TVC (cfu) of the dilution series are presented in Table 1.

ISO 16140³ was used to evaluate equivalence of the two methods. Guidance within the ISO states that data sets closest to 50% positive and 50% negative should be used for evaluation. We therefore assessed equivalence using the 1 in 100 dilution (see Table 2) and determined that they were not equivalent.

However, further statistical investigations with this data set, using non-inferiority analysis, demonstrates that BacT/ALERT is superior to TVC (p=0.003, McNemar test). Table 2 shows that RMM will provide at least 9.82% more positive results than TVC.

Using the dilution to extinction principle, we propose that if a sample is diluted 1 in 10 and demonstrates an absence RMM result then the original sample would contain < 10 cfu/ml. This allows inferences to be made about bioburden using the BacT/ALERT RMM system.

Table 2.

Evaluation of positive results from the two methods of analysis

Dilution	Positive results		% difference	95% CI
	Alternative method RMM	Reference method TVC		
1 in 50	90	82.5		
1 in 100	70	45	25	9.82 to 38.62
1 in 1000	12.5	2.5		

Conclusion

We conclude that BacT/ALERT is superior to TVC when assessing the presence of *S.aureus* at the lower levels of TVC detection.

We therefore propose that RMM can be used as a presence/absence test to determine the dilution extinction point of a contaminant and thus infer a sample bioburden.

Acknowledgment:

Welsh Assembly Government for Pharmacy Practice Development Scheme funding and bioMerieux for technical support

References:

- Hiom S. et al (2010). Investigation into rapid microbial detection methods (RMM) to improve the QA of NHS manufactured aseptic products. *Int. J. Phar. Pract.* **18** (Suppl 2) 92.
- British Pharmacopoeia. Appendix XVI B: Microbiological Examination of Non-Sterile Products. 2009. HMSO: London.
- BS EN ISO 16140:2003 Microbiology of food and animal feeding stuff – protocol for validation of alternative methods.