



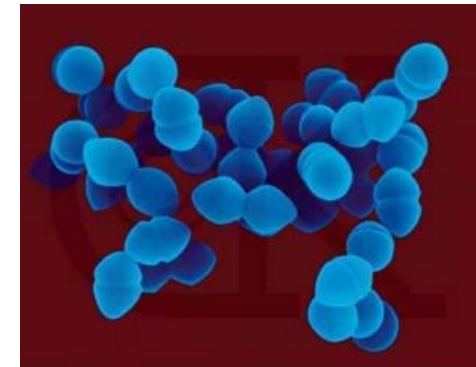
New real-time PCR assay for detecting VRE

Nicole Arbour, Chris Harder, Baldwin Toye, Karam Ramotar

CACMID, February 28th-March 1st, 2008

Vancomycin Resistant *Enterococci* (VRE)

- Plasmid-mediated glycopeptide resistance was first detected in 1986
- VRE is currently the 3rd leading cause of nosocomial bloodstream infections in the USA



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E. faecium

<http://www.buddycom.com/bacteria/gpc/gpcentero.html>

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Vancomycin Resistance Genes

- *VanA* & *VanB* are the most clinically relevant VRE resistance genes.
 - *VanA* shows high levels of resistance to vancomycin (MIC > 32 mg/L) & teicoplanin (MIC > 16 mg/L)
 - *VanB* has variable resistance to vancomycin (MIC 8-512mg/L), but is generally susceptible to teicoplanin (MIC < 2 mg/L)

- *VanC* is a naturally occurring low level intrinsic vancomycin resistance

PCR for Rapid Microbial ID

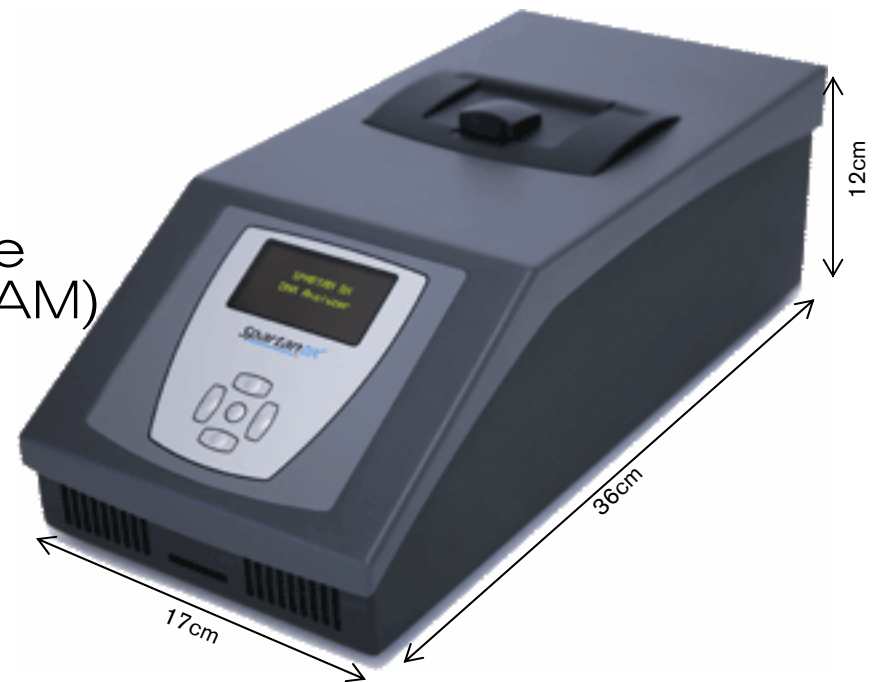
- Culture techniques require a 48-72 hour turnaround time
 - Variability in VanB (MIC 8-512mg/L) makes it challenging to identify.
- Real-time PCR methods have higher sensitivity and reduced processing time



The Spartan DX

- Single channel instrument
- 4 reaction wells
- Two-temperature PCR
- Detection: DNA binding dye (SYBR Green) or probe (FAM)
- Easy to operate
- Low cost
 - MSRP \$9,995

SpartanDX[™]



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BHQ_{plus}TM Probes

- Comparable to LNA[®] or MGB[®] chemistries
- Increased probe T_m
- Shorter probe
- Reduced background fluorescence
- Higher fluorescence



Purpose

To design rapid, efficient and cost effective real-time PCR assays for the detection and genotyping of VRE, for use on the Spartan DX platform

Primer/Probe Design

- *Van A*
 - One set of primers & one probe
 - Recognizes *VanA*
- *Van B*
 - One set of primers & one probe
 - Recognizes *VanB*, *VanB2/3*
- *Van C*
 - One set of primers & two probes
 - Recognizes *VanC1*, *VanC2*, *VanC3*

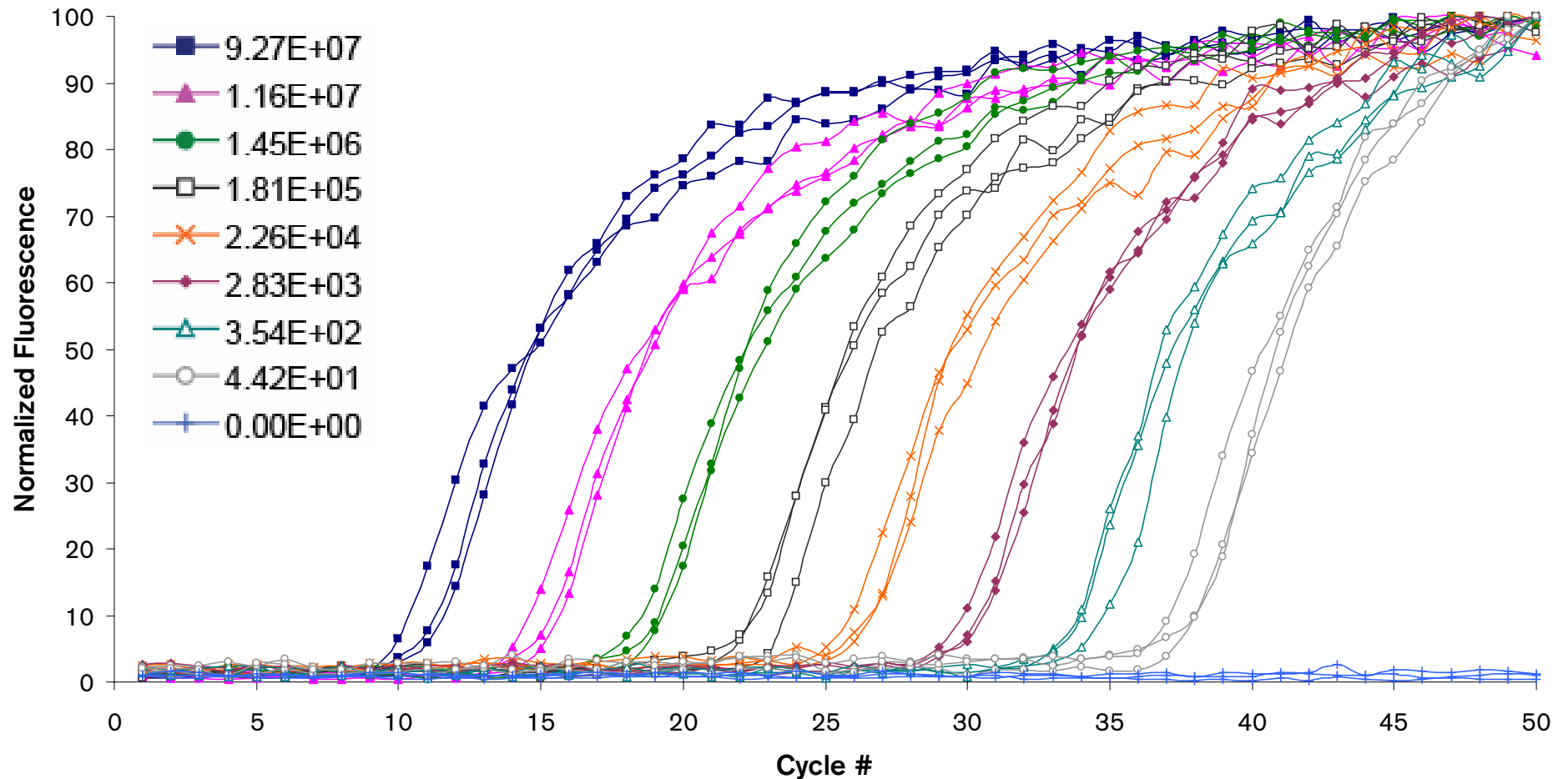
VRE Primer/Probe Sets

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
<i>Van A</i> primers	tat gat ggc cgc tgc agg ta	cgg tga aat tat ccc aag tgg c	163
<i>Van A</i> probe	6-FAM-tgc act tcc cga act g-BHQ+		
<i>Van B</i> primers	gcc atg caa aac cgg gaa ag	caa gcg att tcg ggc tgt ga	92
<i>Van B</i> probe	6-FAM-tgagccacggtatcttc-BHQ+		
<i>Van C</i> primers	ggg aag atg gca gta tcc aag g	gct tga tgc agc agc cat tt	109
<i>Van C1</i> probe	6-FAM-cct tat gtt ggt tgc ca-BHQ1+		
<i>Van C2</i> probe	6-FAM-tgc ctt atg tag gct gc-BHQ1+		

6-FAM = 6-carboxy-fluorescein, BHQ+ = Black Hole Quencher plus

Reaction Efficiency

Estimated Copy Number (*VanB*)



Program Validation

Genotype* (N=70)	Spartan DX		LightCycler
	Van ABC	Van AB	Van AB
VRE <i>Van A</i>	24	24	24
VRE <i>Van B</i>	34	34	34
VRE <i>Van C</i>	11	-----	-----

*Isolates determined as VRE through standard lab culture methods

Cross-reactivity

Isolates [†]	N	Spartan DX	
		Program 1	Program 2
VSE	20	20 (Negative)	20 (Negative)
Non-enterococci	10	10 (Negative)	10 (Negative)

[†]Isolates genotyped using standard culture methods

Non-enterococci tested include:
MRSA, E. coli, MSSA, GCS, GGS

Reproducibility

Sample	Isolate 1	Isolate 2	Isolate 3
Genotype [†]	<i>Van A</i>	<i>Van B1</i>	<i>Van B2/3</i>
N	20	20	15
Average C _T	27.52	29.19	32.75
Std Dev	0.51	1.36	1.29

[†]As determined by the Roche LightCycler *VanA/VanB* Detection assay

Simulated Stool Samples-Procedure

Isolation Method: Boiling Lysis

VRE isolates suspended in saline
(0.5 McFarland = 10^8 CFU/ml)



Serial dilutions
(10^7 , 10^6 , 10^5 , 10^4 , 10^3 , 10^2 , 10^1)



“Spike” with negative “stool swab”



Extract DNA by boiling lysis (10 min)



Run real-time PCR

Isolation Method: ATE Lysis

VRE isolates suspended in ATE
(0.5 McFarland = 10^8 CFU/ml)



Serial dilutions
(10^7 , 10^6 , 10^5 , 10^4 , 10^3 , 10^2 , 10^1)



“Spike” with negative “stool swab”

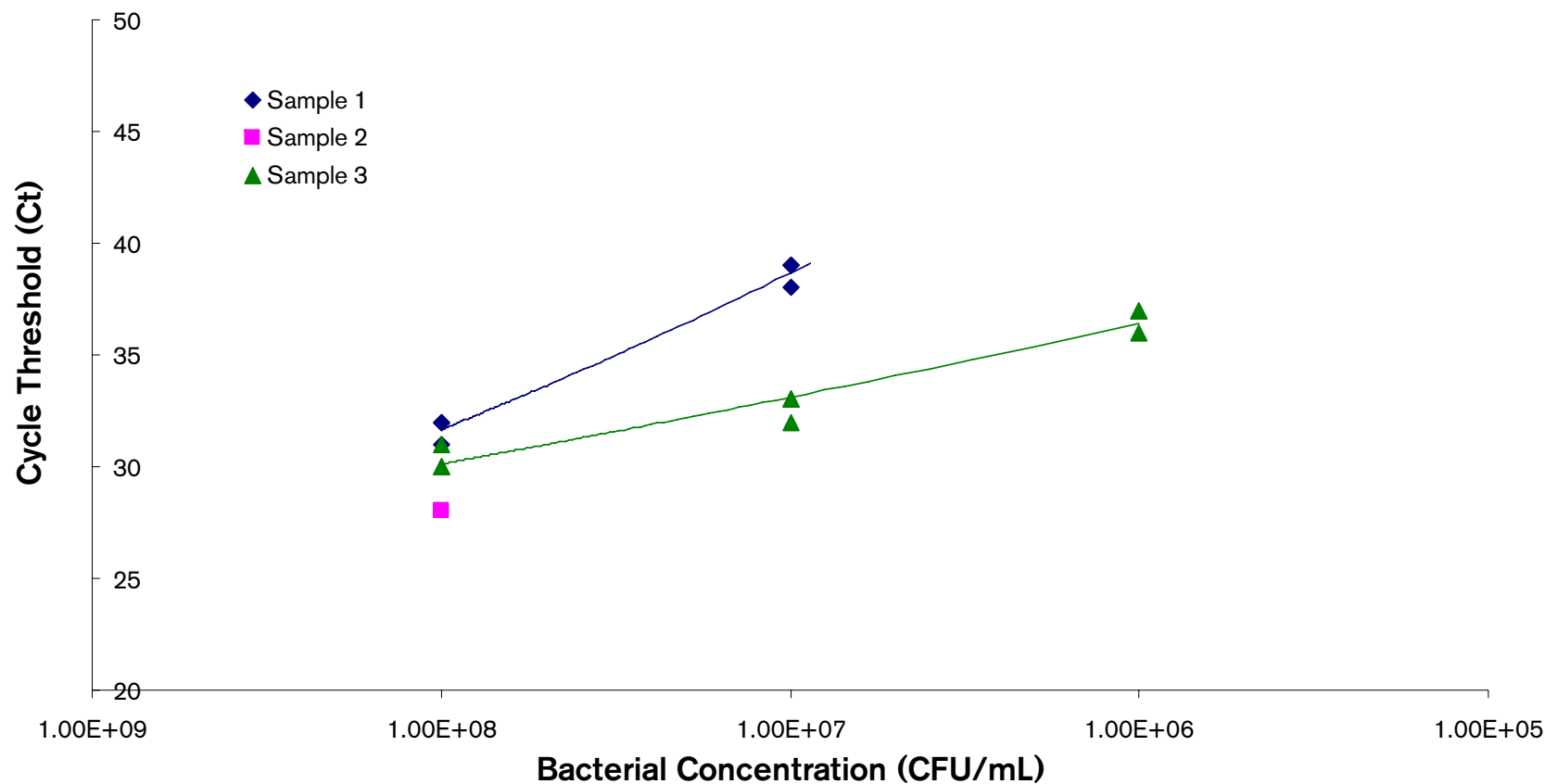


Extract DNA: 10min @ 37°C & boil 10 min
(20min)

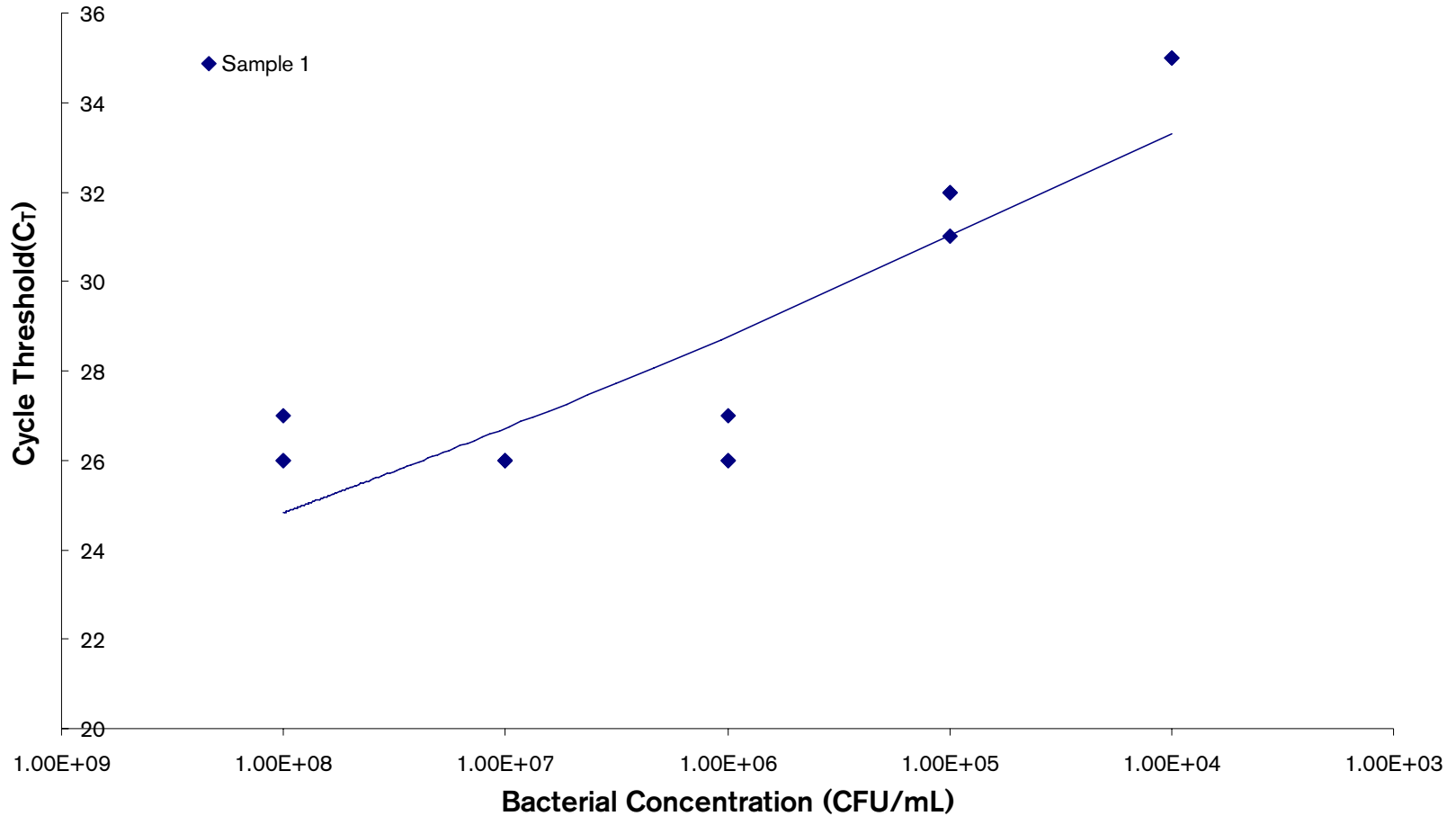


Run real-time PCR

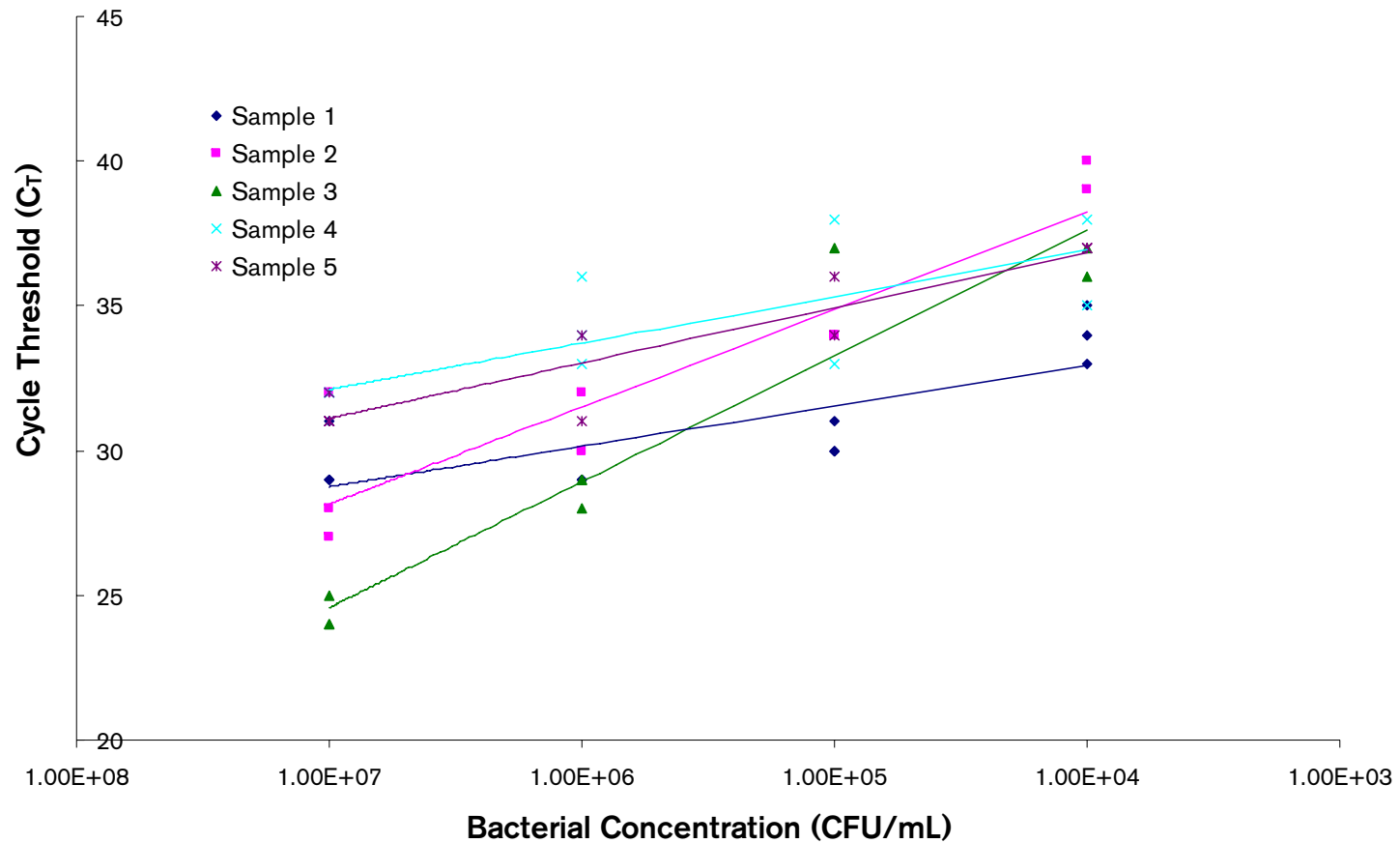
Simulated Stool: Boiling Lysis



Simulated Stool: ATE Lysis



Simulated Stool: ATE Lysis 1/10 Dilution



Conclusion

- Both programs efficiently identify Van A, B, C from colony isolates
- Sensitivity limit $\sim 10^8$ CFU/mL for boiling lysis with simulated stool samples
- Sensitivity limit $\sim 10^6$ CFU/mL diluted ATE lysis with simulated stool samples
- Successful detection of VRE using BHQ_{plus} chemistry from spiked broth

Acknowledgements

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