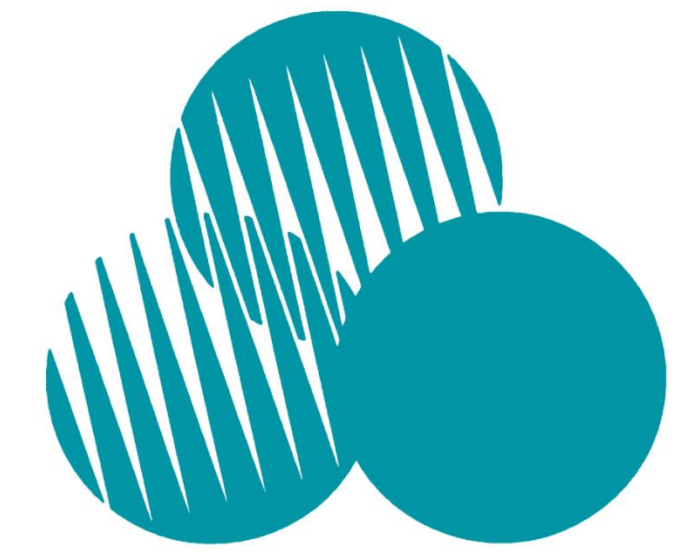


Development of a novel, disposable device to aid in rapid detection of hard-to-lyse organisms associated with Hospital Acquired Infections



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Introduction

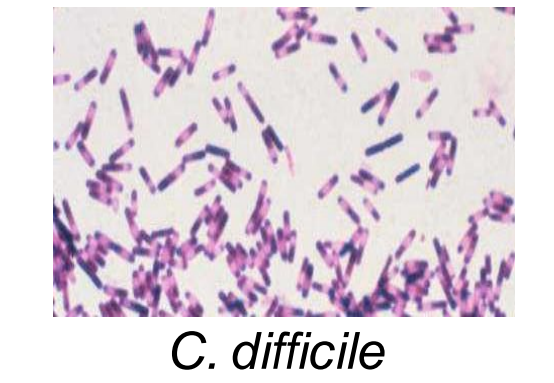
- Project Goal:** Develop a disposable device for the rapid lysis and detection of pathogens associated with Hospital Acquired Infections (HAI)
- Lysis of *Clostridium difficile* and extraction of nucleic acid (NA) extraction can be performed via the PureLyse® technology from Claremont BioSolutions LLC.
- PureLyse® is suitable for other point-of-care (POC) NA detection applications using qPCR and/or isothermal nucleic acid amplification reactions.

Background

Hospital Acquired Infections (HAI)

- Infection not present at the time of admission: bacterial, viral, or fungal
- Clostridium difficile* has surpassed methicillin-resistant *Staphylococcus aureus* (MRSA) as the leading nosocomial infection
- Estimated direct medical costs from HAIs worldwide: \$28-45 billion (CDC, 2009)
- Estimating the Cost of *C. difficile* Infections in the U.S.:

	Cases	Deaths	Annual excess cost
Hospital acquired: Hospital-onset	165,000	9,000	\$1.3 Billion
Hospital acquired: post-discharge	50,000	3,000	\$0.4 Billion
Nursing home - Onset cases	263000	16500	\$2.1 Billion
Total	478,000	28,500	\$3.8 billion

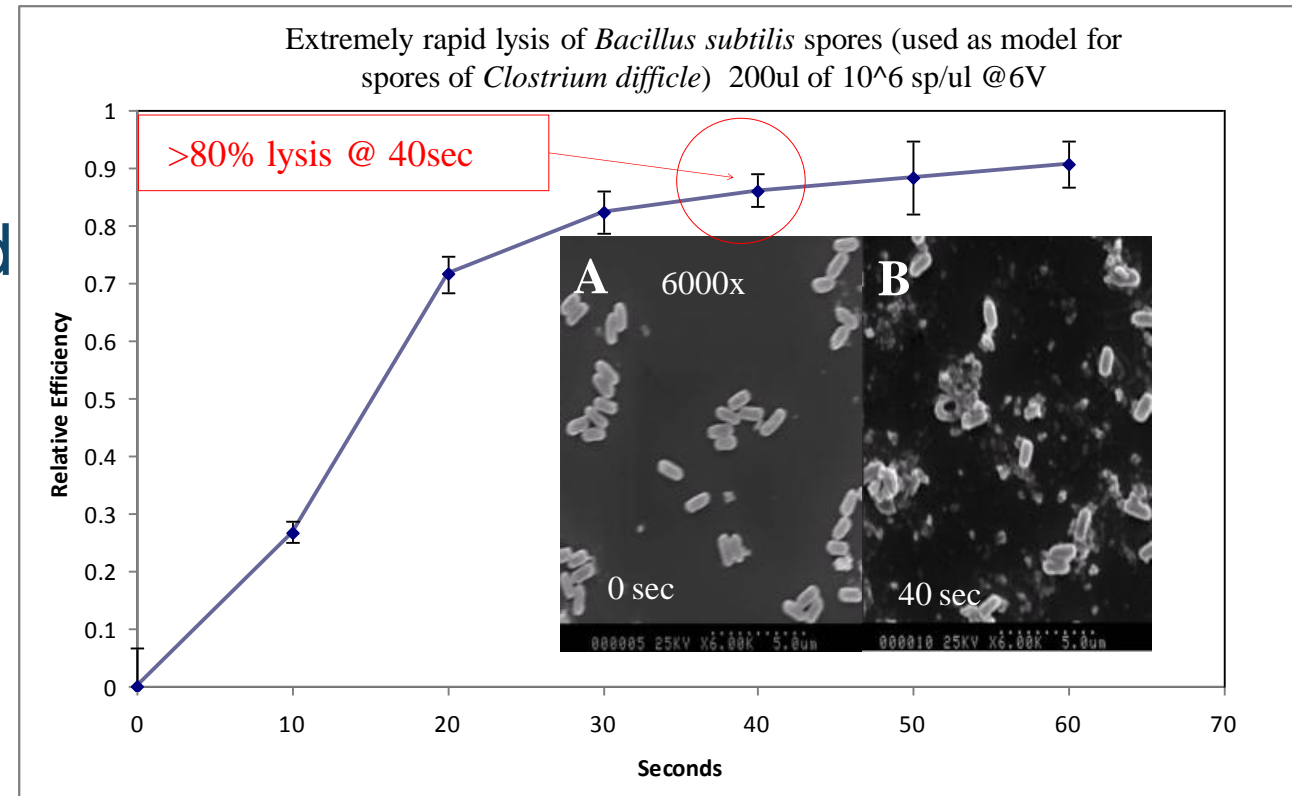


Average excess cost / patient: **\$7878**

Centers for Disease Control and Prevention. (2009). The Direct Medical Costs of Hospital -Acquired Infections in U.S. Hospitals and the Benefits of Prevention. Retrieved from: www.cdc.gov/ncidod/dhqp/pdf/Scott_CostPaper.pdf

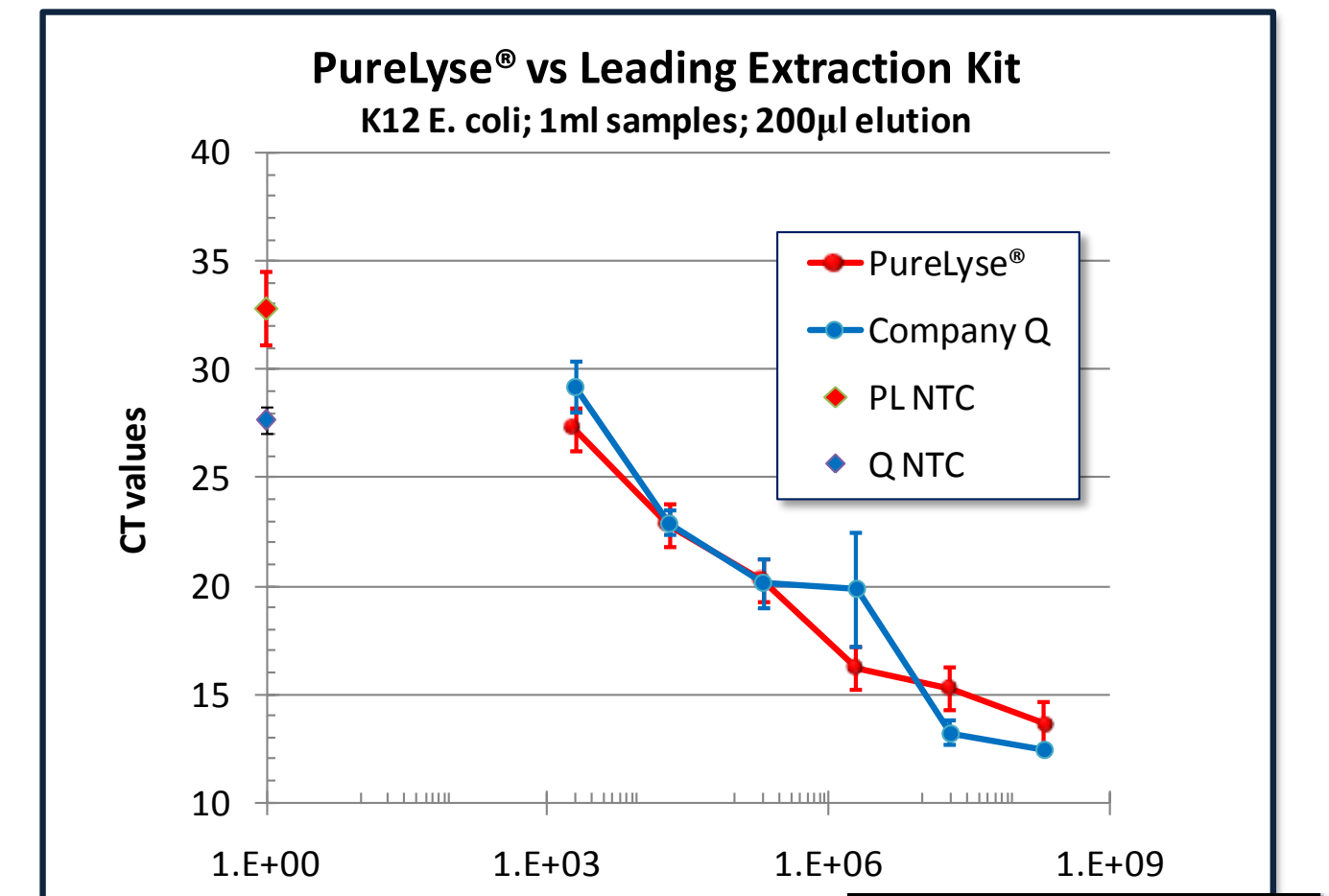
Clostridium difficile Associated Disease (CDAD):

- Clostridium difficile* - Anaerobic spore-forming bacteria
 - Present in normal bacterial flora
- Can cause excessive diarrhea in immunocompromised individuals and antibiotic-treated patients
 - Vegetative and spore cells found in stool
 - Spores can be found on surfaces such as metal and plastic



PureLyse®: Making the Extraction of DNA Easy™

- Fast: 3 to 5 minutes
- Easy to Use: 2 steps
 - Reduce user errors
- Small and disposable, lead-free & RoHS compliant
- No harsh reagents
 - No inhibition of downstream amplification
 - No need for wash steps
 - No harsh reagents disposal ("Green")



Sample: 1 ml of *E. coli*, (n=3)/titer
 Lysis 1 min Elution: 1 min. 200 µl, 2x, 5ul to PCR

CFUs in Sample	Copies in PCR
200,000,000	5,000,000
20,000,000	500,000
2,000,000	50,000
200,000	5,000
20,000	500
2,000	50

C. difficile Diagnostic Market Analysis

Comparison of Current Detection Methods

	Sensitivity	Specificity	Turnaround time	Cost
Toxigenic culture	100%	93-96%	3-4 days	\$22.00
Cytotoxicity assay	80-100%	97%	3 days	\$13.00
GDH antigen screen	70-96%	90-94%	10 minutes	\$17.00
EIA A/B toxin	48-80%	70-96%	25 minutes - 24 hours	\$9.50
PCR	92-100%	90-96%	45 minutes - 3 hours	\$25-50

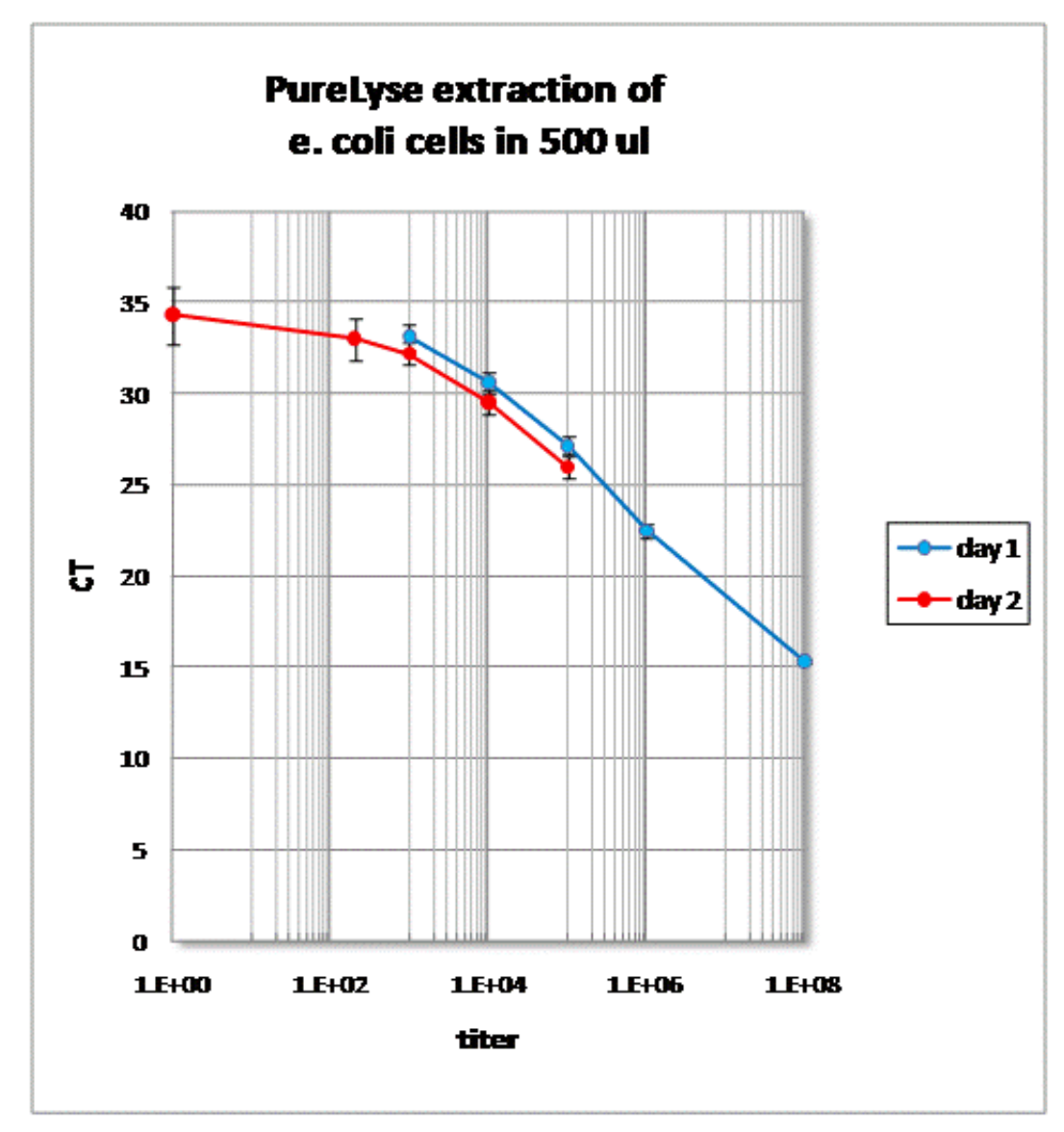
Weaknesses of Current Systems:

- Cepheid GeneXpert: fully-integrated system requiring large investment from hospitals
- Larger hospitals: sampling testing is out-sourced to contract lab testing services
 - Turn around time of 1-5 days, even with PCR methods
- Patients may be released before testing results are received
 - Infected patients must then be readmitted (Cost average \$7878 but can be >\$25,000)

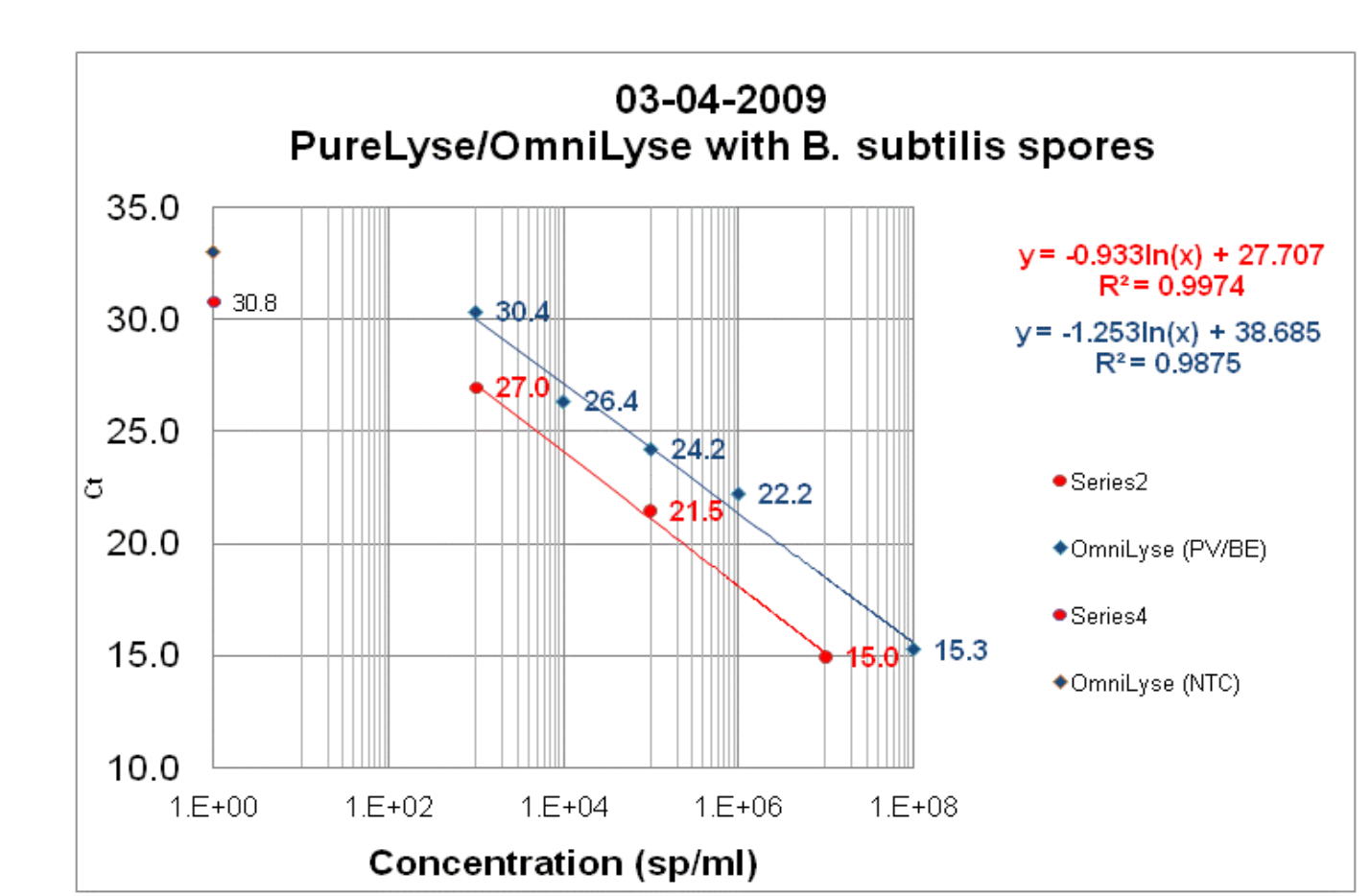
Detection Limit Requirements for C. diff project

- Concentration in infected patient stool: 10⁷ to 10⁴ cells *C. difficile* /gram of stool
- C. difficile* is classified as a BSL2 agent
 - Experiments will be done off site
- Current Model System: *B.subtilis* (BSL1)
 - Genomic DNA, vegetative cells, spores
- Current method of analysis: qPCR
 - Gold standard: Useful backup detection method
 - Quantitative: useful for characterizing PureLyse™ purification method
 - Eventual goal to use public domain isothermal amplification reaction, such as EXPAR, discovered at KGI.

PureLyse® directly into qPCR: bacterial LOD



PureLyse to PCR, *E. coli* titration. Samples were 0.5 ml each. Each titer was tested in triplicate. Titrers on Day 1 were 1000 to 1x10⁸ cells and 200 to 100,000 cells on day 2. Lysis-1.5 min., elute 1 min. in 200 ul. 25 ul transferred to PCR.



Comparison of OmniLyse™ and PureLyse™ titration of *Bacillus subtilis* spores. The OmniLyse™ PCR reactions received 12.5 ul of sample and 37.5 ul of master mix, while PureLyse used 25 ul of sample and 25 ul of master mix. 500 ul of the OmniLyse™ samples were processed. 1000 ul of The PureLyse™ samples were processed and eluted into 200 ul. The final concentration of the master mix components (i.e. MgCl₂, BSA, etc) were the same. The thermocycling profiles were the same. Sample concentrations of 1,000, 100,000 and 100,000,000 cells were tested.

Sample Matrix Requirements

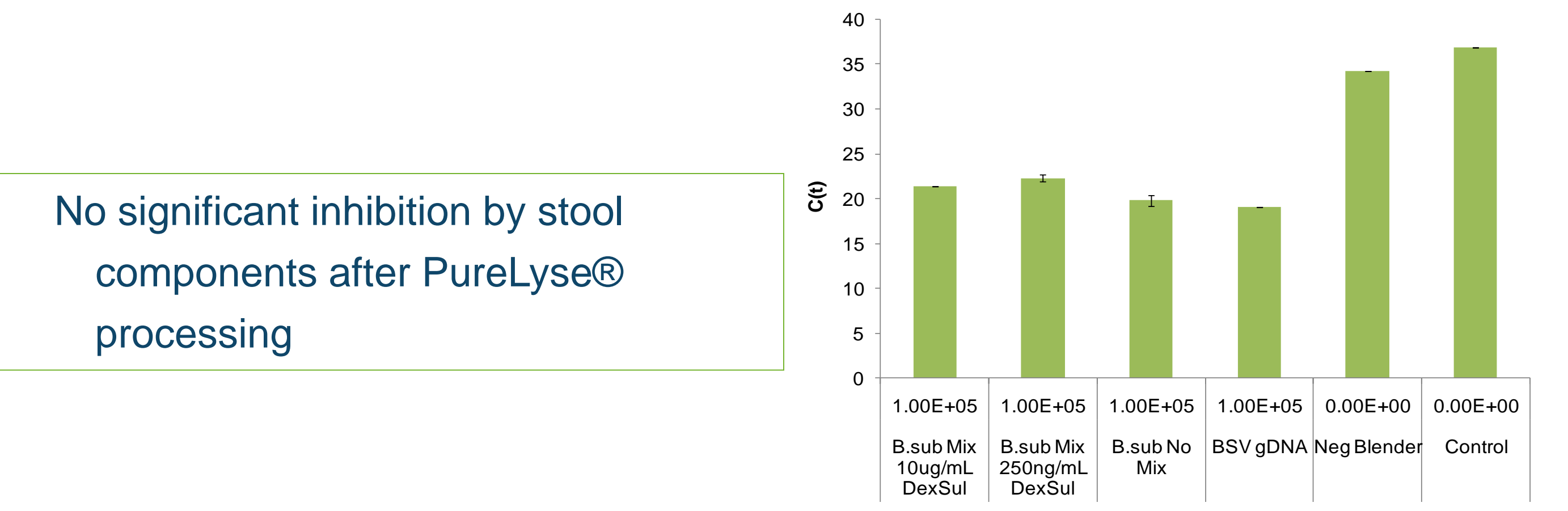
Sample Matrix: Fake Stool "Cocktail"

- Stool sample components inhibitory to PCR
 - Proof-of-concept testing for PureLyse™ and integrated cartridge
 - Test components separately, then "cocktail"
- Major potential interfering components:
- Bile Salts
 - Mucin
 - Human Serum Albumin
 - Anionic Polysaccharides
 - Dextran sulfate used as model

Bile Salts and Mucin do not significantly inhibit PCR after PureLyse™ processing.

- HSA seems to promote binding (Ct values lower than genomic DNA control)
- High levels of dextran sulfate inhibit PCR, whereas low concentrations do not

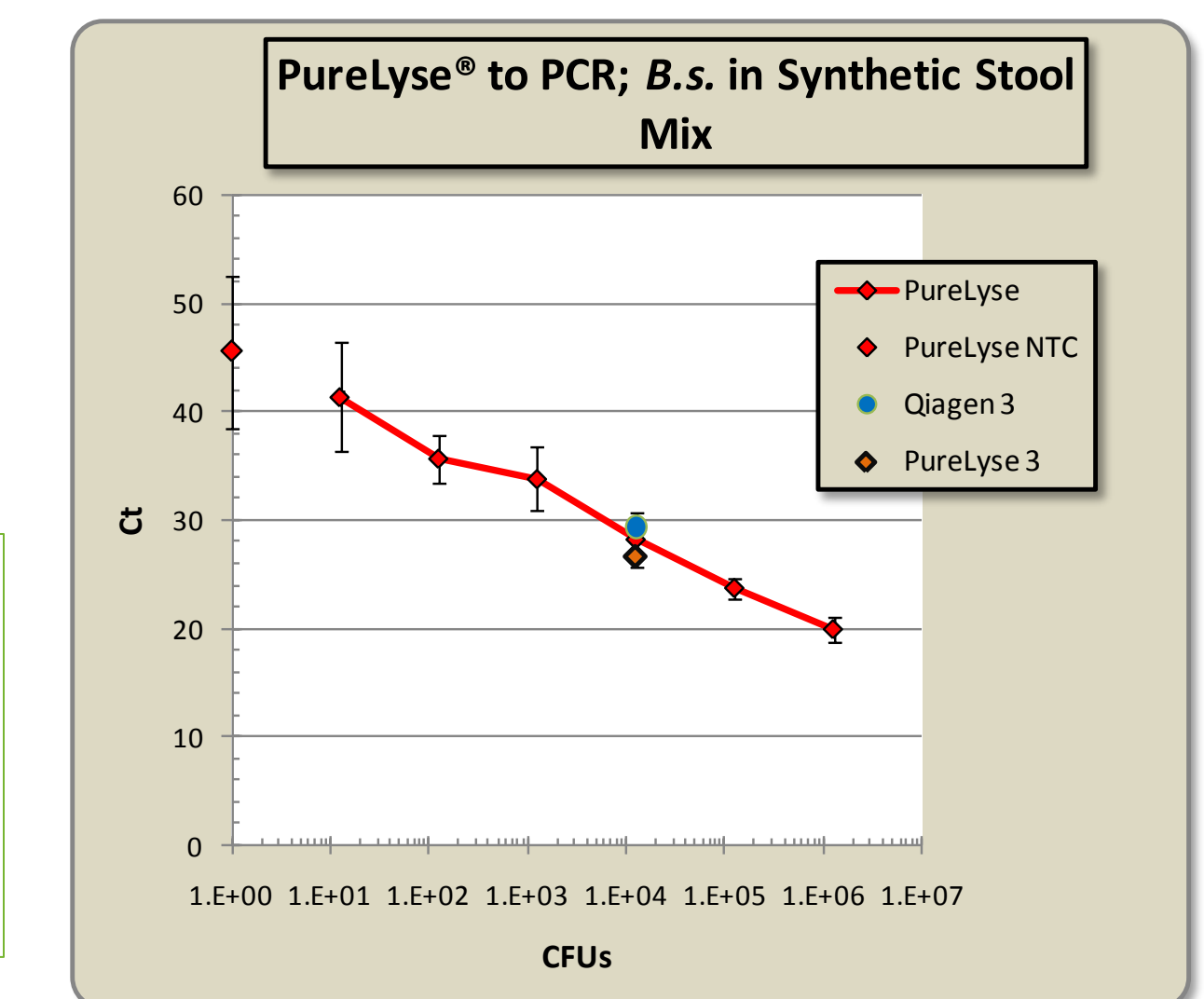
PureLyse® processing of B. subtilis vegetative cells to PCR



No significant inhibition by stool components after PureLyse® processing

Next study: Human gDNA, 2ugm and *E. coli* cells, 1x10⁸ were added to the Synthetic Stool Cocktail

Synthetic Stool Mix
 Dextran Sulfate
 Bile Salts
 Mucin, Bovine
 Human Serum Albumin
 Human gDNA
E. coli



Copies in PCR	CFUs in PureLyse	Replicates
0	0	6
12.5	100	6
125	1000	6
1250	10000	5
12500	100000	5
125000	1000000	5
1250000	10000000	5

100 µl Synthetic stool samples brought up to 1 ml in Binding Buffer
 • Lysis - 2 minutes
 • Elution -1 minute, 200 µl
 • 25 µl into PCR

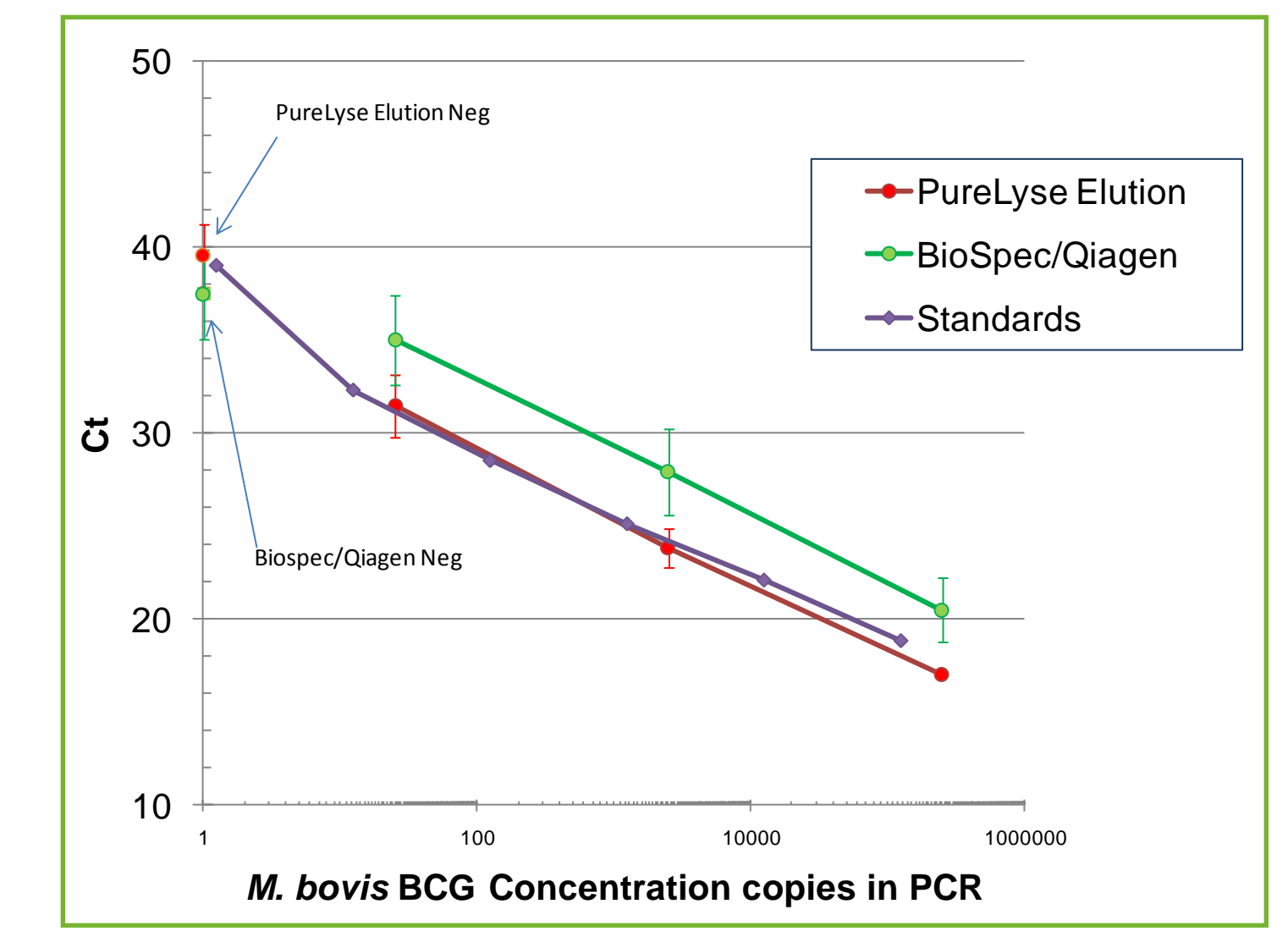
Conclusion: ClaremontBio's PureLyse® technology offers an opportunity for rapid detection of *C. difficile* for POC market

In another effort: success w/ M bovis as model for Mtb

PureLyse®: Mycobacterium bovis BCG

PureLyse® samples:
 •500 µl lysis and DNA capture - 3 minutes
 •250 µl elution

Biospec samples:
 •625 µl lysis Biospec bead beater - 3 minutes
 •500 µl processed via Qiagen DNeasy Kit
 •250 µl elution



	CFUs/ml	copies in PCR
Purelyse	100	2.5
	200	5
	1000	25
	100000	2500
	10000000	250000
biospec	200	6.25
	1000	31.25
	100000	3125
	10000000	312500
std curve	100	1.25
	1000	12.5
	10000	125
	100000	1250
	1000000	12500

12.5 µl into PCR

Data provided by Peter Vandeventer and Angelika Niemi, Keck Graduate Institute

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