

Development of a rapid 15-minute test using Glycan-functionalised latex nanoparticles for identification of Uropathogenic *Escherichia coli* (UPEC)

M. Kyriakides¹, JB. Vendeville¹, J. Preece¹, H. Newman¹, A. Mauleon Amieva¹, M. Holbrow-Wilshaw¹, S. Medina-Villar¹, J. Samphire², Y. Takebayashi³, J. Ramos-Soriano², N. Dorh¹, J. Spencer³, M. Carmen Galan², J. Dorh¹

¹ FluoretiQ, Unit DX, St Phillips Central, Albert Road, Bristol, BS2 0XJ

² School of Chemistry, University of Bristol, Cantocks Close, Bristol, BS8 1TS

³ School of Cellular & Molecular Medicine, Biomedical Sciences Building, Bristol, University of Bristol, BS8 1QU

INTRODUCTION

- Urinary tract infections (UTI) are one of the most frequent infectious diseases worldwide, affecting 150 million people every year. [1]
- Uropathogenic *Escherichia coli* (UPEC) strains are responsible for >80% of all UTI cases. [2]
- Accurate determination of UTIs relies on 48-hour cultures, which leads to over-prescription of broad-spectrum antibiotic, contributing to the global threat of antimicrobial resistance. [3-4]
- A novel 15-minute agglutination assay (NANOPLEX) was developed that utilises image processing software to enable detection of UPEC at clinically relevant concentrations.

METHOD

The NANOPLEX assay:

- Carboxylate modified latex microspheres functionalised with Heptyl alpha-D-mannoside using EDC coupling or copper-catalysed azide-alkyne cycloaddition (7CM)
- Functionalised latex microspheres incubated with *E. coli* at room temperature for 15 minutes

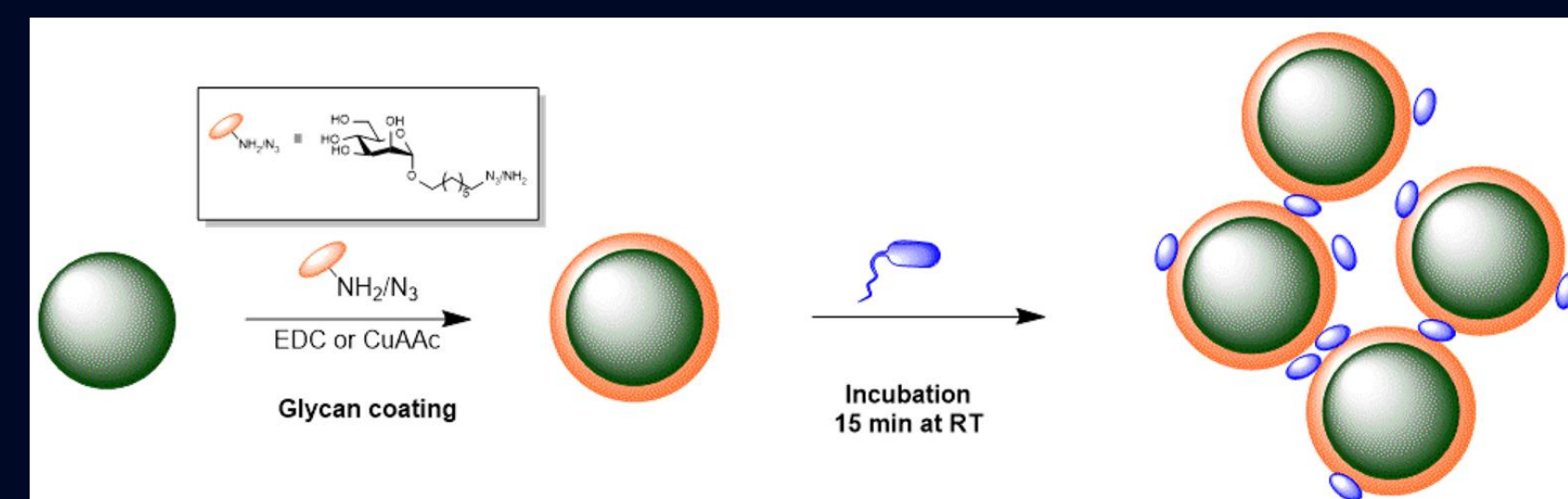
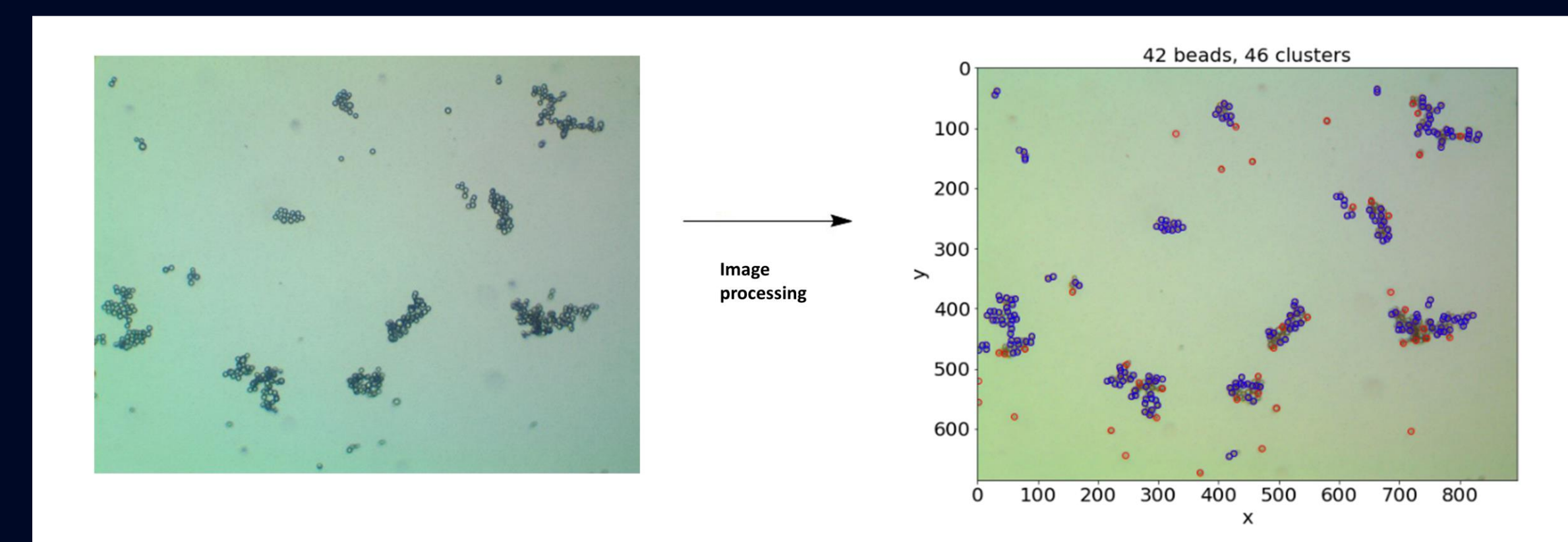


Image processing:

- Agglutination events imaged using bright-field microscopy
- Image processing software tracks agglutination events, identifying clusters vs single beads.
- Total cluster size (TCS) calculated = Sum of beads in all genuine clusters identified in a sample



RESULTS

SAMPLE IMAGES

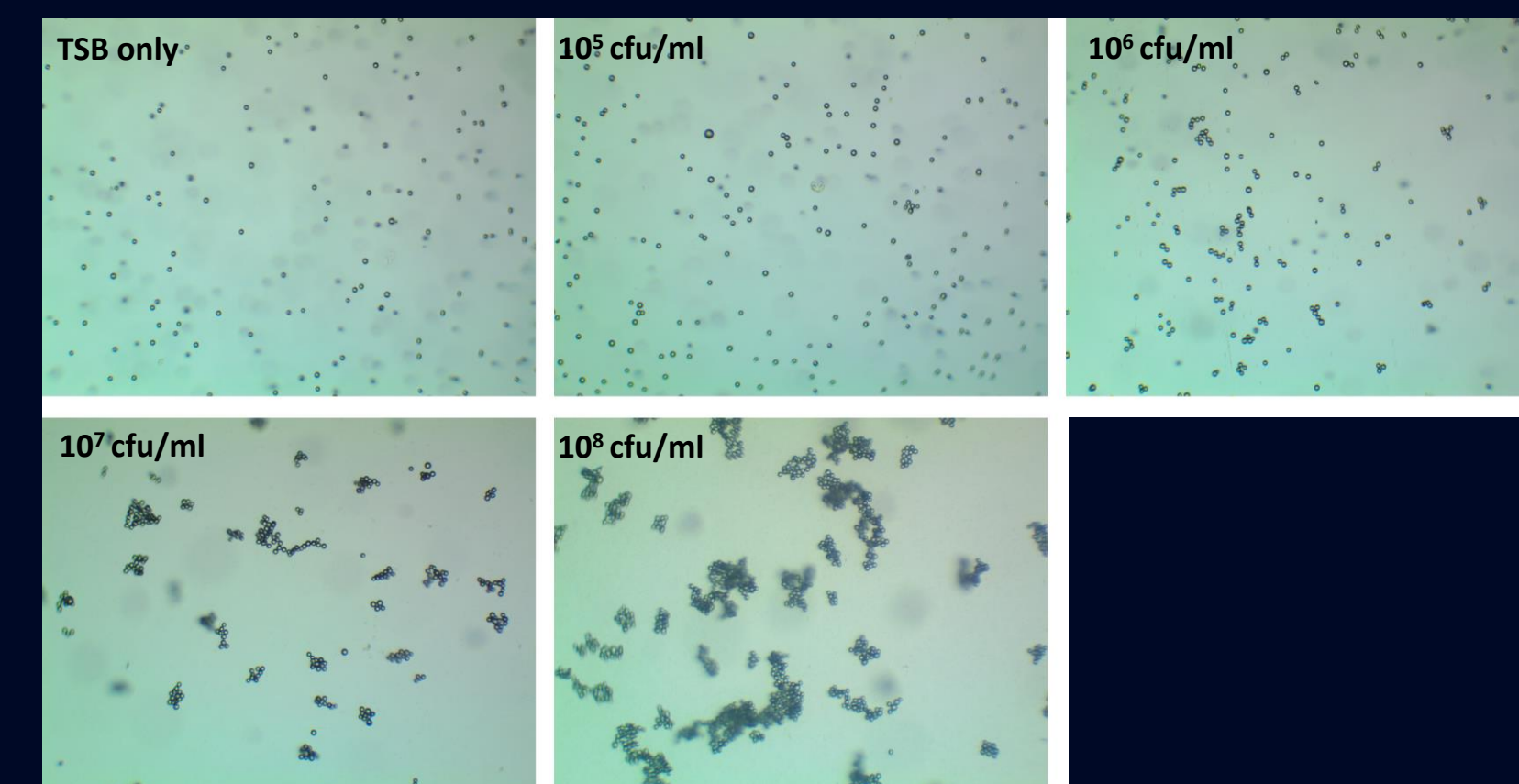


Figure 1: Images of 7CM & *E. coli* BW25113 ($10^5 - 10^8$ cfu/ml) in TSB

STRAIN TO STRAIN VARIABILITY

As NANOPLEX and the image processing software were developed on *E. coli* lab strain BW25113, we wished to determine variability between uropathogenic strains.

E. coli strains NCTC 9001 and 12241 were both grown in TSB and standardised to 10^8 CFU/ml and tested using the NANOPLEX™ Assay.

Comparable TCS signals were observed across strains tested.

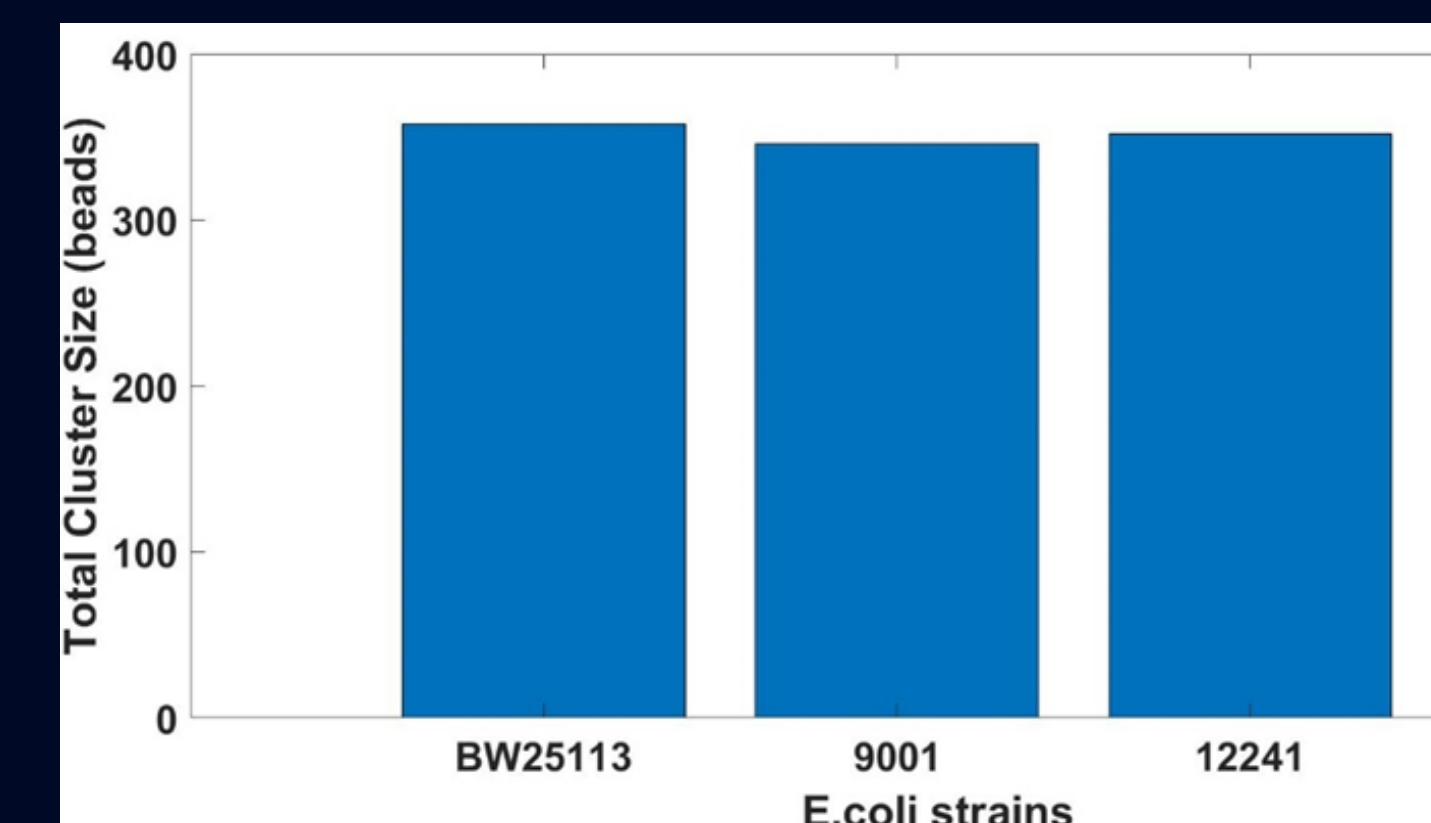


Figure 2: TCS comparison of *E. coli* strains BW25113, NCTC 9001 and NCTC 12241 at 10^8 cfu/ml

CONCLUSIONS

- NANOPLEX assay identified and enumerated UPEC using 7CM-conjugated latex microspheres and an image processing software, that correlates agglutination events to bacteria load.
- 100x concentration of *E. coli* prior to NANOPLEX allowed the detection of clinically relevant bacterial concentrations, sensitive to 10^3 cfu/ml.
- Future work will focus on detection of UPEC and enhancement of TCS signals in clinical human urine.

PERFORMANCE IN TSB (10^5 - 10^8 CFU/ML)

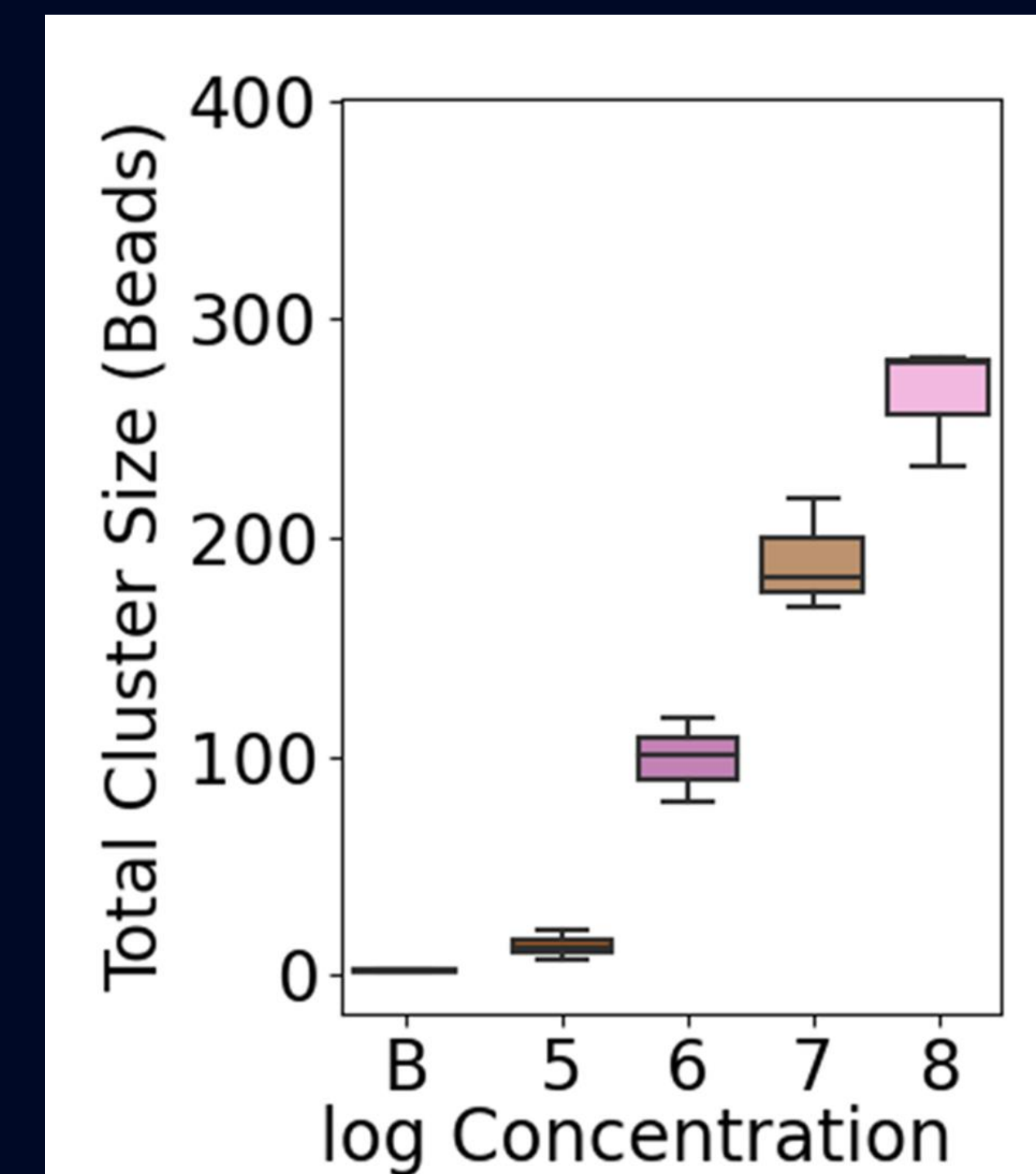


Figure 3: The relationship between 7CM & *E. coli* BW25113 ($10^5 - 10^8$ cfu/ml) in TSB

A range of concentrations of *E. coli* BW25113 in TSB incubated with 7CM conjugated latex microspheres was investigated.

A positive correlation in TCS signal was observed from $10^5 - 10^8$ cfu/ml. The average 10^5 cfu/ml signal was approximately 8-times higher than the negative control of sterile TSB. Also a narrow spread is observed each successive log concentration of *E. coli*

PERFORMANCE IN HUMAN URINE (10^5 - 10^8 CFU/ML)

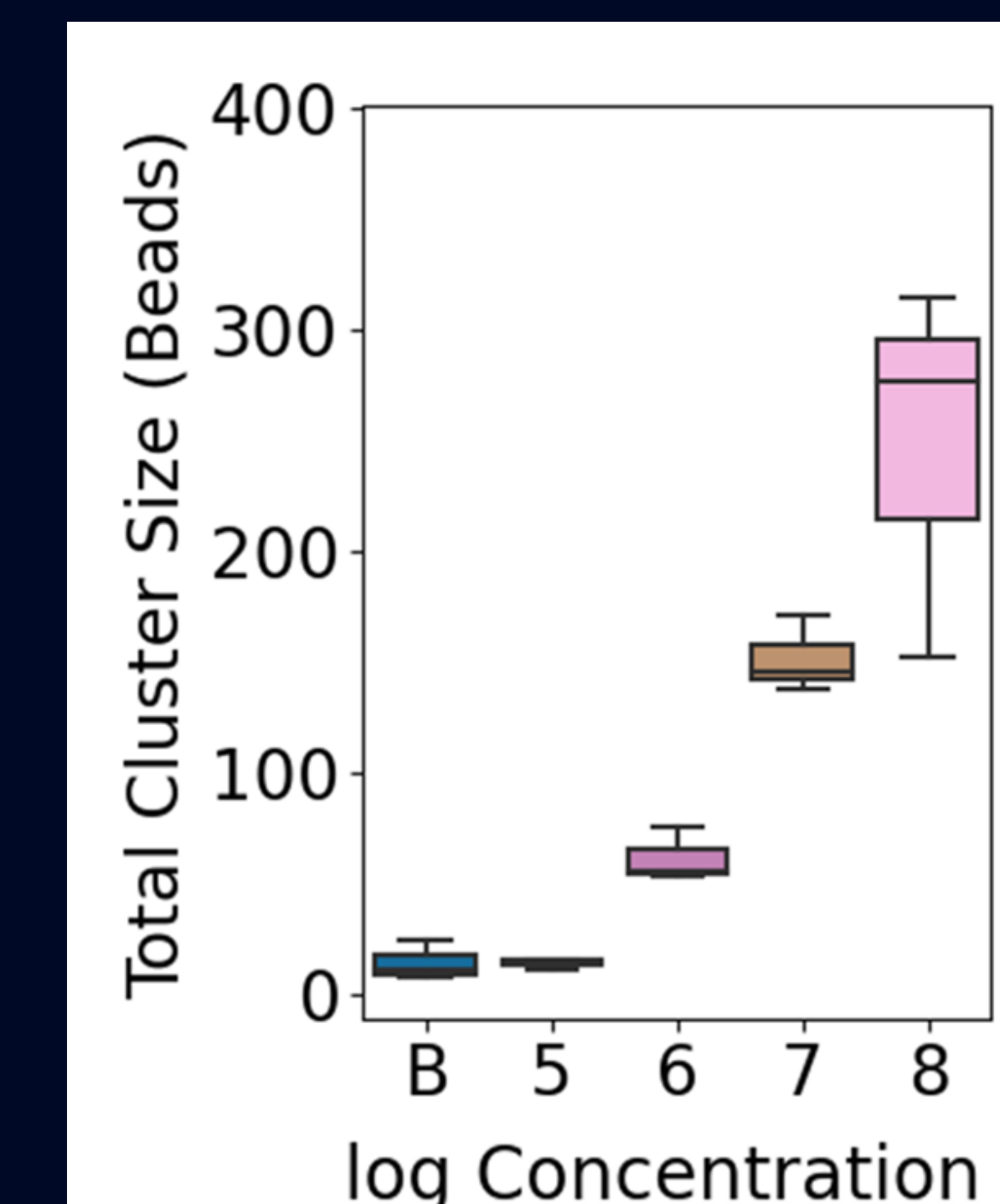


Figure 4: The relationship between 7CM & *E. coli* BW25113 ($10^5 - 10^8$ cfu/ml) in human urine

The performance of the assay in identifying bacteria in human urine was investigated. $0.2 \mu\text{m}$ filtered mid-stream urine inoculated with *E. coli* BW25113 (grown overnight in TSB) to 10^5 to 10^8 cfu/ml, and incubated with 7CM.

A positive correlation in TCS values upon bacterial concentration was observed. TCS signals were on average 23% lower at 10^6 to 10^8 cfu/ml than their respective values in TSB alone.

$0.2 \mu\text{m}$ filtered urine negative control had a TCS higher than 10^5 cfu/ml samples, which brought the limit of detection up to 10^6 cfu/ml. Also a greater spread was observed at 10^8 cfu/ml compared to samples in TSB.

EXTENDING LOWER LIMIT OF DETECTION (10^3 - 10^8 CFU/ML)

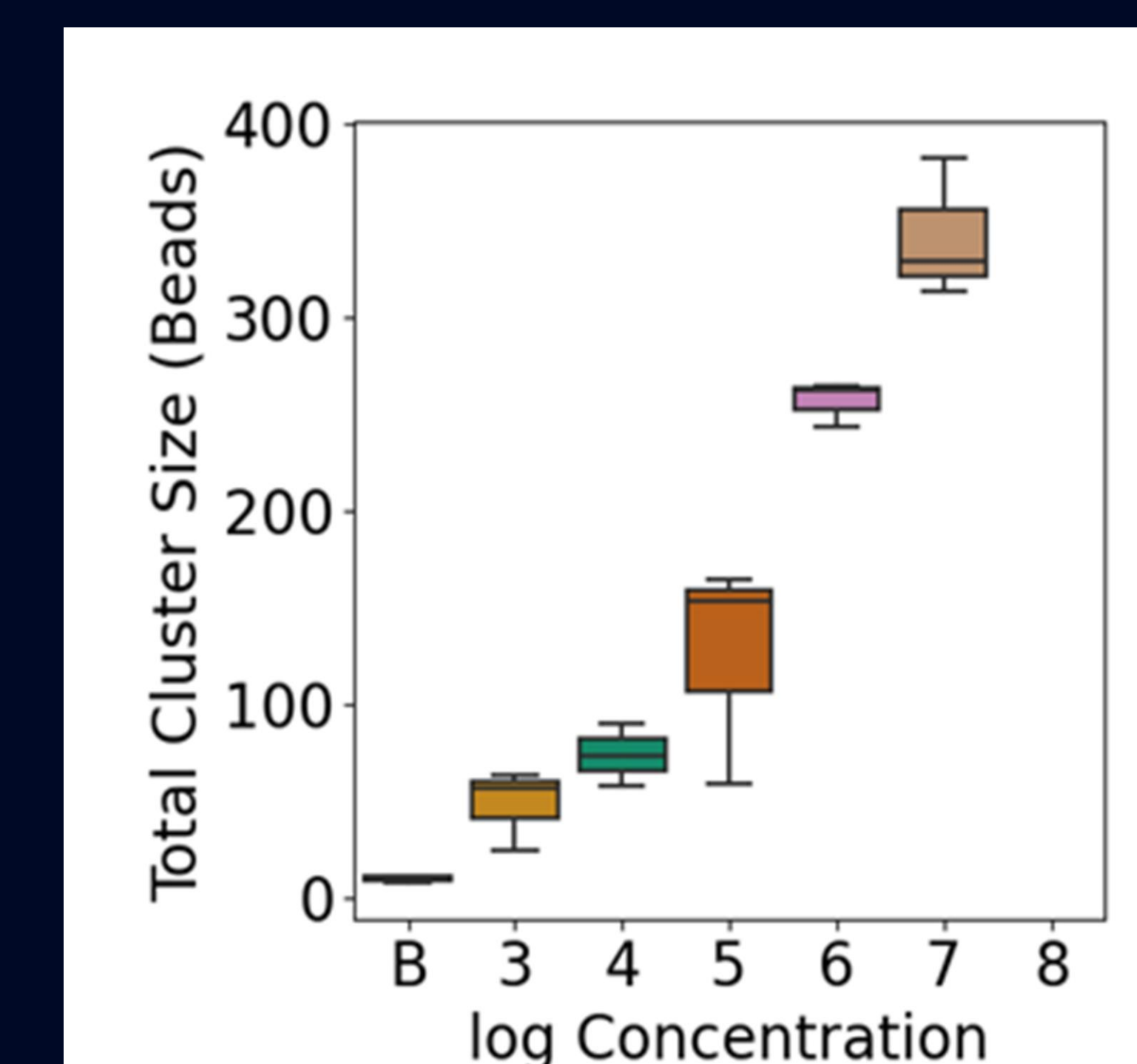


Figure 5: The relationship between 7CM & 100x concentrated *E. coli* BW25113 ($10^5 - 10^8$ cfu/ml) in TSB

UK standards for microbiology state that bacterial counts between 10^2 and 10^5 cfu/ml may, depending upon specific patient groups, be clinically significant for UTI diagnosis. [5-7] Therefore, optimization of the NANOPLEX assay was needed in order to detect UPEC at these levels.

To improve the sensitivity of the assay, a 100-fold concentration step was carried out prior to carrying out the NANOPLEX assay with *E. coli* BW25113 in TSB.

After concentration, there was a demonstrable enhancement in the TCS signal, extending the sensitivity of the assay to 10^3 cfu/ml.

REFERENCES

- Data presented is from the paper: Vendeville, J-B; Kyriakides, M; Preece, J; Samphire, J; Takebayashi, Y; Ramos-Soriano, J; Mauleon-Amieva, A; Holbrow-Wilshaw, M; Newman, H; Medina-Villar, S; Dorh, N; Spencer, J; Dorh, J; Galan, M. Fast identification and quantification of uropathogenic *E. coli* through computer generated cluster analysis. *ACS Biomater. Sci. Eng.* Due to be published in 2021.
- [1] Flores-Mirales, A. L.; Walker, J. N.; Caparon, M.; Hultgren, S. J. Urinary Tract Infections: epidemiology, mechanisms of infection and treatment options. *Nat. Rev. Microbiol.* **2015**, *13*, 269-284.
- [2] Barber, A. E.; Norton, J. P.; Spivak, A. M.; Mulvey, M. A. Urinary Tract Infections: Current and Emerging Management Strategies. *Clinical Practice.* **2013**, *57*, 719-724.
- [3] Nace, D. A.; Drinka, P. J.; Crichton, C. J. Clinical uncertainties in the approach to long term care residents with possible urinary tract infection. *J. Am. Med. Dir. Assoc.* **2014**, *15*, 133-1339.
- [4] Infectious Diseases Society of America. The 10 × 20 initiative: pursuing a global commitment to develop 10 new antibacterial drugs by 2020. *Clin. Infect. Dis.* **2010**, *50*, 1081-1083.
- [5] National Collaborating Centre for Women's and Children's Health (UK). Urinary Tract Infection in Children: Diagnosis, Treatment and Long-term Management. London: RCOG Press; **2007**. (NICE Clinical Guidelines), 54.
- [6] Stamm, W. E.; Counts, G. W.; Running, K. R.; Fiho, S.; Turck, M.; Holmes, K. K. Diagnosis of Coliform Infection in Acutely Dysuric Women. *N. Engl. J. Med.* **1982**, *307*, 463-468.
- [7] Kunin, C. M. A Reassessment of the Importance of Low-Count Bacteriuria in Young Women with Acute Urinary Symptoms. *Ann. Intern. Med.* **1993**, *119*, 454.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support from the ERC Consolidator Grant: 648239, the Global Challenges Research Fund EP/T020288/1, and the EPSRC funds: EP/G036764/1, EPSRC EP/S026215/1 and EP/R043361/1 (TS/R014329/1). The authors are also grateful for the use of the characterization facilities at University of Bristol and for their help in characterizing the compounds.

CONTACT INFORMATION

For all further enquiries, please contact j.preece@fluoretiq.com.