

Glycan functionalisation of latex nanoparticles for detection of Uropathogenic *Escherichia coli* (UPEC) species



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1. Introduction

Urinary Tract Infections (UTI) present an unrelenting burden on public health as one of the most common bacterial infections and are the second highest use of antibiotic prescriptions in primary care [1]. There is an ominously rising trend of antibiotic resistance in urinary pathogens [2] and even with new drugs under development, the only way to safeguard the future is to develop diagnostic tools that allow us to rapidly identify bacteria at the point of consultation.

Uropathogenic *Escherichia coli* (UPEC) strains are responsible for >80% of all UTI cases yet accurate determination of infection is still reliant on the 48-hour microbial culture, causing many benign cases of bacteriuria to develop into recurrent GP visits, long term antibiotic use and hospitalisation [3-5].

Uroepithelial cells are coated in glycans that allow cell to cell communication and interaction with their surrounding environment. All bacteria have evolved glycan binding proteins which recognise these sugars, and pathogens exploit this mechanism for adherence to establish an infection [6].

We have developed a novel approach- NANOPLEX™ that uses synthetic glycan-coated latex nanoparticles to mimic the glycan-binding interaction between host and pathogen. Using NANOPLEX™ probes and simple microscopy we were able to identify clinically relevant UPEC species and concentrations.

2. Assay Overview

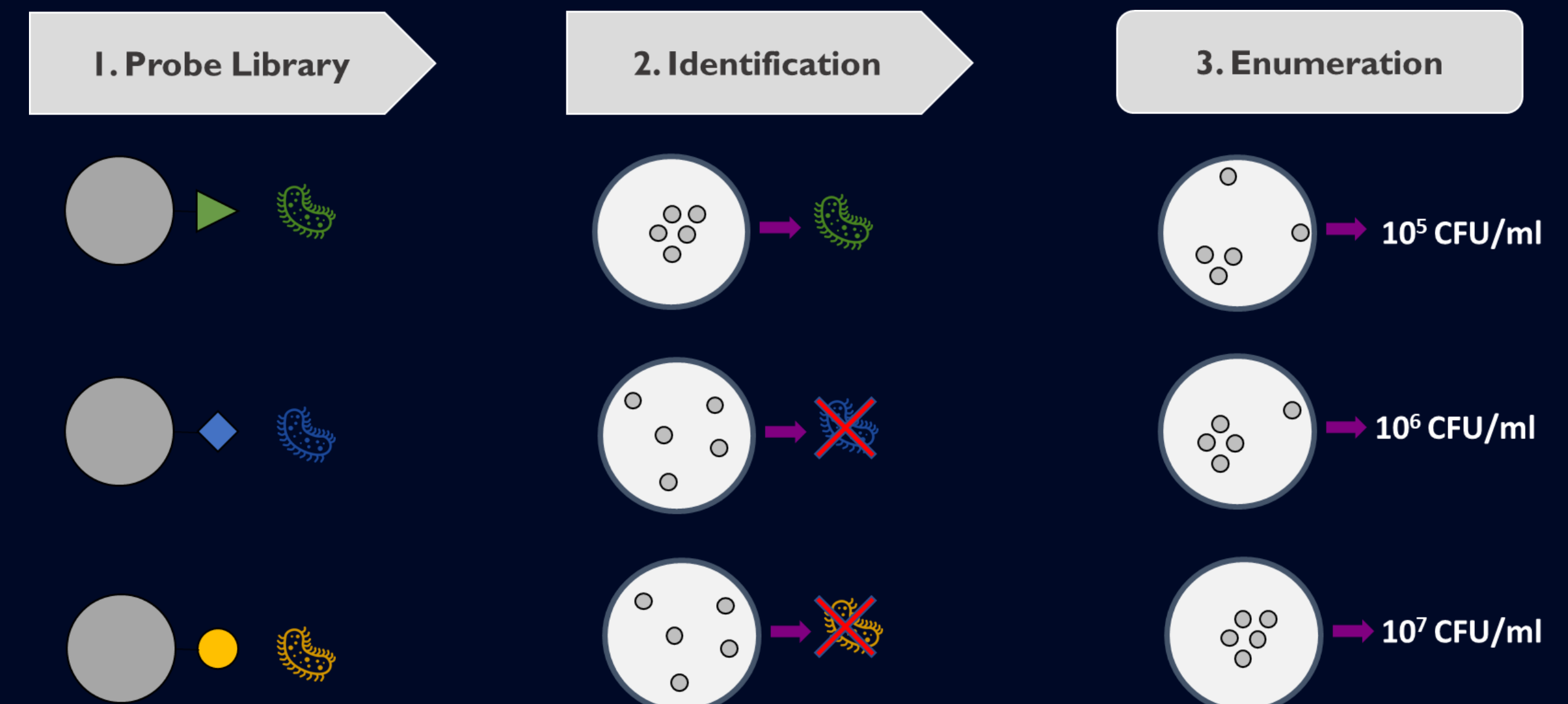


Figure 1. Schematic overview of NANOPLEX™ principle

3. Method

Synthesis of NANOPLEX™ probes

Mannose moieties and carbon-chain linkers of various lengths were conjugated to Molecular probes™ CML Latex Beads, 4% (w/v) and fluorescent carbon dots (FCD) using EDC coupling.

Bacterial Strains

Overnight Cultures of *E. coli* BW25113 and BW25113ΔfimA were grown in Tryptic Soya Broth (TSB) and standardised to 10⁷ CFU/ml.

NANOPLEX™ assay

Agglutination is the clumping of microspheres and typically achieved using antibody epitope: paratope interactions. We have developed an alternative approach that exploits the glycan: lectin interaction via induction of a multivalent catch bond and selective agglutination of UPEC species in 15 minutes.

Sample prep

1. Pipette *E. coli* sample into Eppendorf.
2. Add NANOPLEX™ probe.
3. Vortex mix the probe and bacteria sample briefly at Room Temperature
4. Vortex mix for 5mins
5. Rest for 10mins at Room Temperature
6. Image 3x

Image Processing

Matlab was used to process the images collected. The single free-floating beads are identified separately to the clusters. The single beads are circular: width and length approximately the same and diameter of 10 μm. A cluster is defined as a group of beads close together which form a non-circular feature.

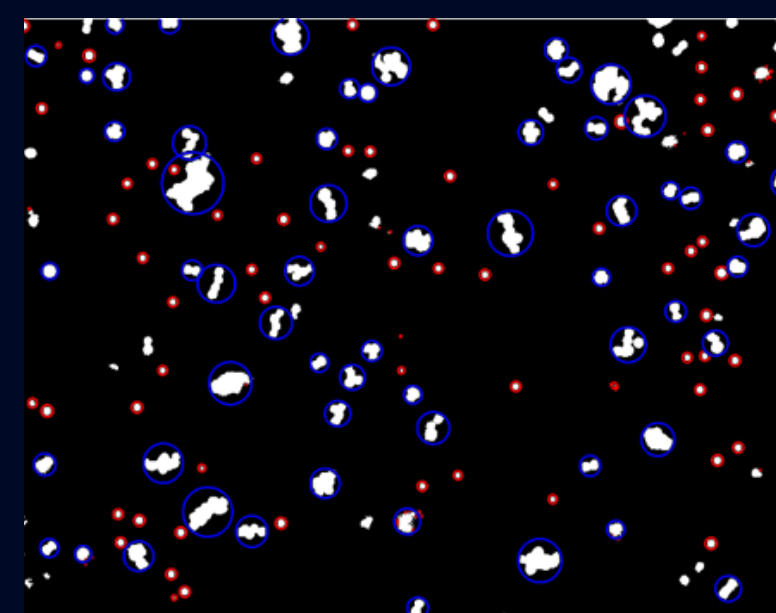


Figure 2. Reconstructed image of sample without bacteria (left) and UPEC species (right)

Validation of Mannose moieties and *E.coli* Fimbriae target

Type-1 pillated bacteria such as FimH producing *E. coli* cause aggregation of mannan-containing *Saccharomyces cerevisiae* by lectin specific interactions [7]. To validate our molecular target we modified a known competitive binding assay [8-9] mannosylated carbon dot nanoparticle and an *E.coli* BW25113ΔfimA mutant to demonstrate that the glycan binding interaction is D-Mannose and FimH dependent.

4. Results

NANOPLEX™ assay

We were able to observe agglutination of NANOPLEX™ probes with *E.coli* BW25113 at 10⁷ CFU/ml in TSB. As demonstrated in Figure 3 we were able to analyse microscopy images to identify the presence and frequency of clusters in the sample containing UPEC species versus the blank sample.

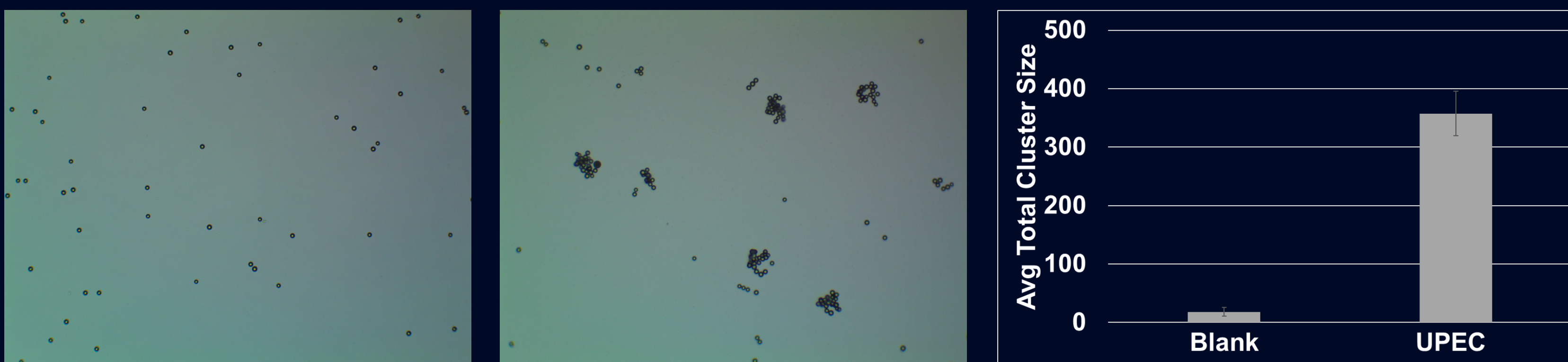


Figure 3. Representative 10x bright-field microscopy image of sample without bacteria (left) and UPEC species (middle). Summary of data n=6 (right)

Validation of Mannose moieties and *E.coli* Fimbriae target

We were able to demonstrate the inhibition of agglutination in *S. cerevisiae* using a Mannosylated carbon nanoparticle. Inhibition of the agglutination in *E. coli* BW25113 and mannosylated FCD's was comparable to the *E. coli* BW25113ΔfimA strain confirming the fimbriae were the glycan-binding protein involved in this binding reaction.

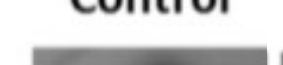
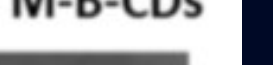
				Control Sample	Mannosylated FCD
	Control	M-B-CDs			
BW25113					
BW25113ΔfimA			BW25113	Full agglutination	No agglutination
			BW25113ΔfimA	No agglutination	No agglutination

Figure 4. Summary of agglutination results obtained for *S. cerevisiae* and *E.coli* strains with and without presence of Mannosylated FCD's

5. Conclusions

- We have validated the use of a glycan-based agglutination assay to detect the most frequently reported uropathogen in UTI.
- The absence of agglutination in *E. coli* BW25113 ΔfimA and yeast cells confirms the binding is Fimbriae dependent and specific to the Mannose moieties conjugated on the surface of the NANOPLEX™ probes.

6. Future Work

- Achieve linear detection of *E. coli* from 10³-10⁸ CFU/ml in TSB and clinical urine samples.
- Expanding the library of NANOPLEX™ probes to cover 95% of pathogens implicated in Urinary Tract Infection
- Single blind studies with clinical urine samples to obtain diagnostic performance indicators (Sensitivity, Specificity, PPV and NPV)
- Translation of the assay into a bespoke hardware and cartridge system for clinical use as a point-of-care test

7. References

- [1] Flores-Mireles AL, Walker JN, Caparon M and Hultgren SJ. (2015). Urinary Tract Infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*. 13 (5) p269-284
- [2] Rudrajit P (2018). State of the Globe: Rising Antimicrobial Resistance of Pathogens in Urinary Tract Infection. *Journal of Global Infectious Diseases*. 10(3) p117-118
- [3] Barber AE, Norton JP, Spivak AM and Mulvey MA. (2013). Urinary Tract Infections: Current and Emerging Management Strategies. *Clinical Practice*. 57(5) p719-724
- [4] Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, and Hummers-Pradier E. (2010). The Diagnosis of Urinary Tract Infection. *Deutsches Ärzteblatt International*. 107(21) p361-367
- [5] Davenport M, Mach KE, Dairiki Shortcliffe LM, Banaei N, Wang TZ and Liao JC. (2017). New and Developing diagnostic technologies for urinary tract infections. *Nature Reviews Urology*. 14(5) p296-310
- [6] Ghorasi AC, and Kohler JJ. (2019). Not all Quiet on the Sugar Front: Glycan Combatants in Host-Pathogen Interactions. *Biochemistry*. DOI: 10.1021/acs.biochem.9b00524
- [7] Ofek I, Mirelman D and Sharon N. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. *Nature* 265, p623-625
- [8] Hultgren SJ, Schwan WR, Schaeffer AJ and Duncan JL Regulation of production of type 1 pili among Urinary Tract Isolates of *Escherichia coli*. *Infection and Immunity*. 54, p613-620
- [9] Mirelman D, Altmann G and Eshdat Y. Screening of bacterial isolates for mannose-specific lectin activity by agglutination of yeasts. *Journal of clinical microbiology*. 11, p328-331