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Development of an Affimer-based hybrid assay for Clostridium difficile infection diagnosis

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Background: *Clostridium difficile* is one of the leading causal agents of hospital acquired infection, and antibiotic-associated diarrhoea. The treatment and control of *Clostridium difficile infection* (CDI) is critically dependent on accurate laboratory diagnosis. However, current diagnostic methods have limitations including cost, potential over-sensitivity and lack of detection of Toxin protein associated with nucleic acid amplification techniques, inadequate sensitivities and specificities of immunoassays and long turnaround time for toxigenic cultures. To date, no one-step diagnostic that is low cost, sensitive and specific is available for CDI diagnosis. This study describes the improved sensitivity and specificity obtained by switching one of the molecular recognition elements of a clinically used *C.difficile* detection kit from antibodies to Affimers.

Material/methods: Leeds has developed a non-antibody binding protein called Affimer type II. From phage display libraries, Affimer binders against >300 targets have been identified with increasing scope for applications in diagnostics, imaging, therapeutics and drug discovery. Here we used Affimers selected by phage display technology in combination with a clinical test kit. Selected Affimers were characterised for specificity, sensitivity, binding kinetics and thermostability. To test for improved sensitivity and specificity of a commercially available clinically used diagnostic kit, a hybrid assay was developed using Toxin B Affimers as capture molecules and the kit conjugate-antibody for detection.

Results: Phage display screening yielded high affinity Affimers against the three well-established biomarkers of CDI (Toxin A, Toxin B and glutamate dehydrogenase). In this study Toxin B Affimers

were used and show no cross-reactivity to Toxin A. The Affimers were expressed in *E. coli*, with soluble protein yield as high as 300 mg/L. Surface plasmon resonance data revealed K_D values for two Toxin B binders of 4.06 nM and 7.48 nM. These binders were monomeric, with thermostability of >80^{o}C with no aggregation. Through sandwich phage ELISA, two Toxin B Affimers have been established for use as a pair in sandwich assay format. The developed Toxin B hybrid assay using one of these as capture reagent showed a higher sensitivity and specificity compared to the kit. The Affimer-antibody combination showed 100% increase in sensitivity at 10 ng/ml Toxin B, similarly there was a 10-fold increase in the limit of detection for the hybrid assay compared to the clinical kit. The kit does not discriminate between Toxin A and B and shows better detection of Toxin A whereas this hybrid assay shows high specificity and sensitivity for Toxin B.

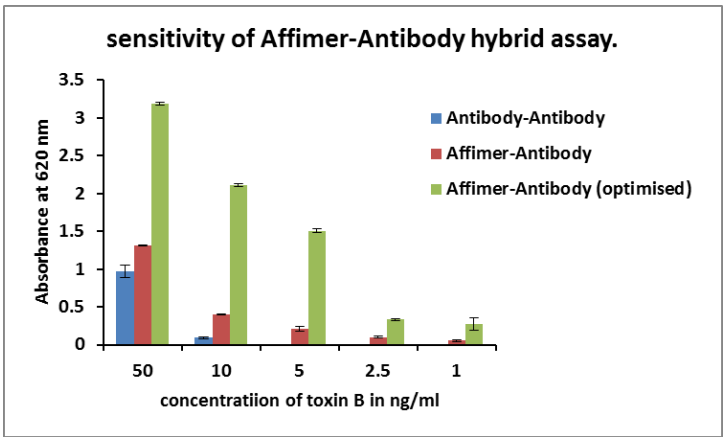

Figure 1: Effect of using Affimer as capturing molecule on the sensitivity and cross-reactivity of a clinically used *C.difficile* detection kit . The comparison of the sensitivity of an Affimer-Antibody hybrid assay using standard protocol (red bar) and optimised protocol (green bar) with Antibody-Antibody assay (blue bar) is presented.

Conclusions: This study suggests that the limitations in the sensitivity of antibody-based ELISA kit used in CDI diagnosis is directly dependent on sensitivity of the molecular recognition element used. Development of an Affimer-based hybrid assay for Toxin B provides a step towards the use of Toxin-based assay as stand-alone tests for CDI diagnosis