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

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Introduction

- ***Clostridium difficile* infection**
- A leading causal agent of hospital acquired infection.
- $12 billion global cost annually

Hospitals known as *a "place of care"*

Now seen as

"place of contracting infection"

➢ **Target of interest** : Toxin A and Toxin B

➢ The treatment and control of *Clostridium difficile infection* (CDI) is critically dependent on accurate laboratory diagnosis.

➢ However, current diagnostic methods have limitations highlighted below

Toxigenic culture

68-90% sensitivity
24-72h
Two gold standard
Laboratory intensive

Immunoassays

24-82% sensitivity
> 84% specificity

Molecular Based assays

$55 cost
Laboratory intensive
potential over-sensitivity
Can't detect toxin protein

➢ CDI is strongly associated with the presence of toxins

➢ Detection of toxin diagnoses clinically important CDI

Objective

To improve the sensitivity and specificity of immunoassays by switching one of the molecular recognition elements of a clinically used *C.difficile* detection kit from antibodies to Affimers.

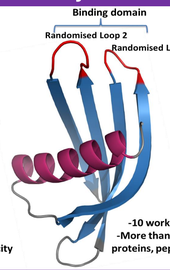
Why Affimer?

Simple
Single chain, no S-S

Extreme stability
-Up to 100 °C
-Stable in a broad pH range

Very small
About 12 kDa

Easy to engineer
- Insertion of cysteines
- Multimers for multispecificity



High affinity
Nanomolar or single digit picomolar

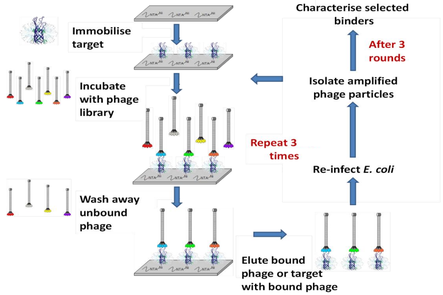
Controlled specificity

High yields
-In *E. coli*
-Cost effective scale-up

Fast selection
-10 working days using Phage display
-More than 450 successful screens against proteins, peptides and chemical compounds

Methods

1. Phage Display screening.



2.Affimer selection

- Phage ELISA: selection of Affimers with high sensitivity and specificity
- DNA sequencing to identify sequence diversity and conserved motifs

3. Protein Expression and purification

- selected Affimer clones were sub-cloned into expression vector
- Protein expression was induced with IPTG
- One-step protein purification with NI-NTA affinity chromatography

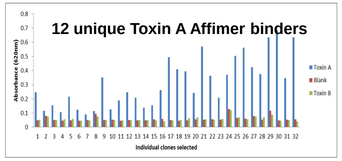
4. Characterisation of selected Affimers

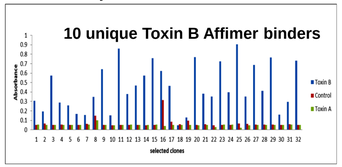
- Cross-reactivity
- Affinity
- Selection of Affimer pairs
- Aggregation profile
- Limit of detection
- Thermostability
- Development of Hybrid immunoassay

Results

1. Selection of Affimers:

Phage Display screening identified specific Affimers against Toxin A with no cross-reactivity to Toxin B and vice versa in phage ELISA assay



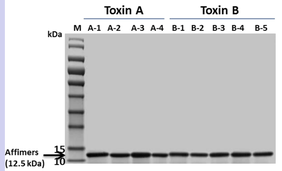


2. Protein purification and biophysical characterisation of Affimers:

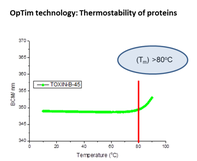
Affimers purified from *E. coli* by Ni-NTA chromatography yielding up to 300 mg/ L.
Surface plasmon resonance: K_D values for two Toxin B binders of 4.06 nM and 7.48 nM.
Affimers are monomeric, thermostability >80ºC and no aggregation at this temperature.

3. Identification of Affimer pairs.

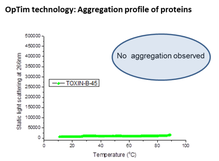
By sandwich phage ELISA, two Toxin B Affimers have been identified as a pair in sandwich assay format.
Limit of detection for the Affimer pair in sandwich phage ELISA was determined.



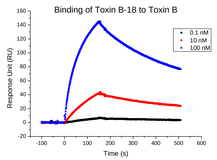
SDS-PAGE Analysis of purified Affimers



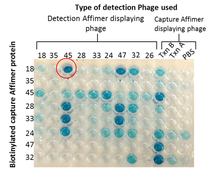
Affimers possess high thermostability



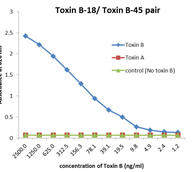
No aggregation displayed up to 90 ºC



SPR shows the affinity of selected Affimers to target



Identification of Affimer pairs with sandwich phage ELISA

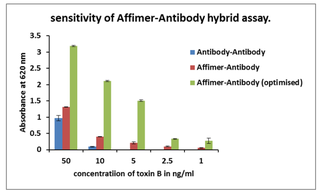


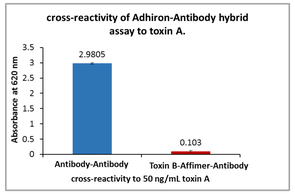
Limit of detection for Affimer pair

Results continued

4. Development of Toxin B hybrid assay:

- Explored enhancing sensitivity and specificity compared with 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.





- **Increased sensitivity**: 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.
- **Increased specificity:** The kit does not discriminate between Toxin A and B with better detection of Toxin A. The hybrid assay shows highly specific detection of Toxin B with no cross-reactivity to Toxin A.

Conclusion

➢ Affimers are robust non-antibody binding molecules for use in point of care microbial diagnostics.

➢ Replacing capturing antibody with Affimer improved the sensitivity and specificity of an ELISA kit against Toxin B

➢ 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.

➢ This is a step towards developing Affimer-only CDI point-of-care diagnostics.

References

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2. TIEDE, C., et al. 2014. Protein Engineering Design and Selection. 27, 145-155
3. POLLOCK, N. R. 2016. Journal of Clinical Microbiology, 54(2), 259–264.

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