Development and application of anti-immune complex antibodies for detection of aflatoxin M1

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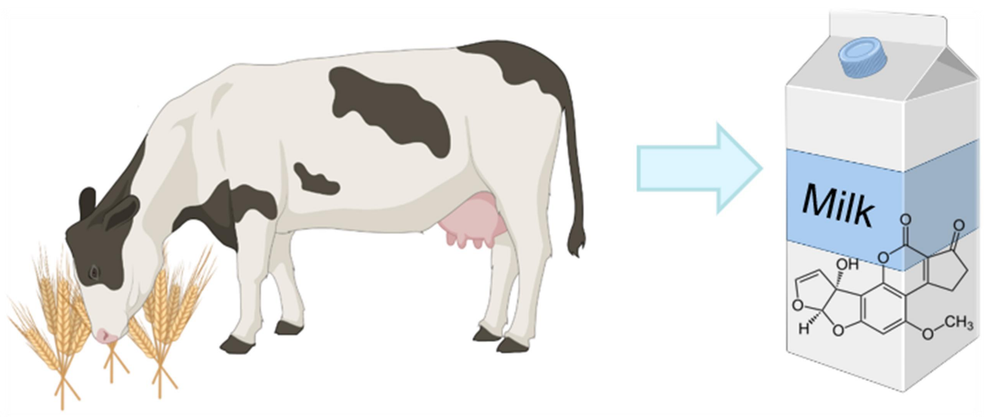
Introduction

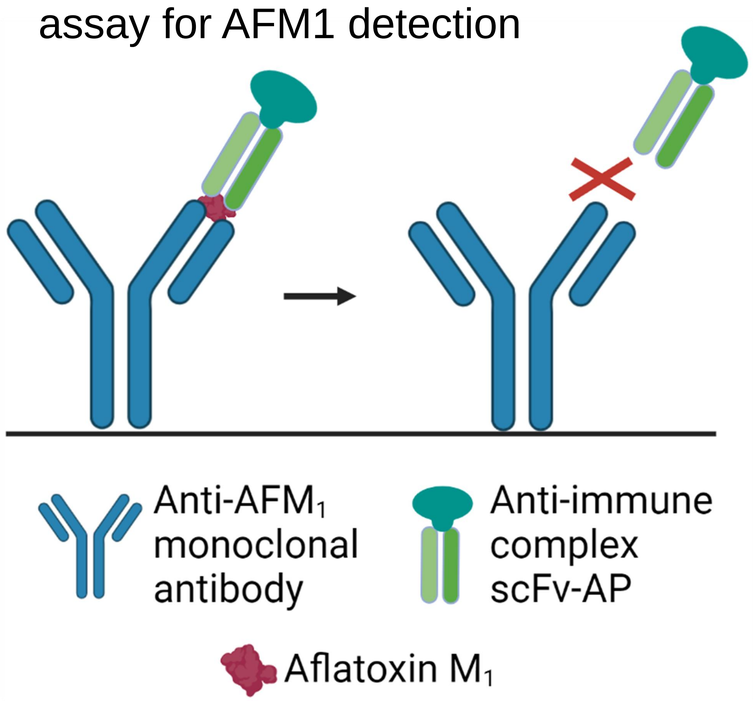
Aflatoxins are mycotoxins produced by fungi *Aspergillus flavus* and *A. parasiticus.* These are small toxic and carcinogenic compounds that contaminate crops, vegetables and fruits causing significant global impact on health and economy. Aflatoxin M1 (AFM1) can be found in milk or milk products derived from livestock that have eaten contaminated feed.

Currently used chromatographic methods for AFM1 quantification are sensitive but generally time-consuming and expensive.

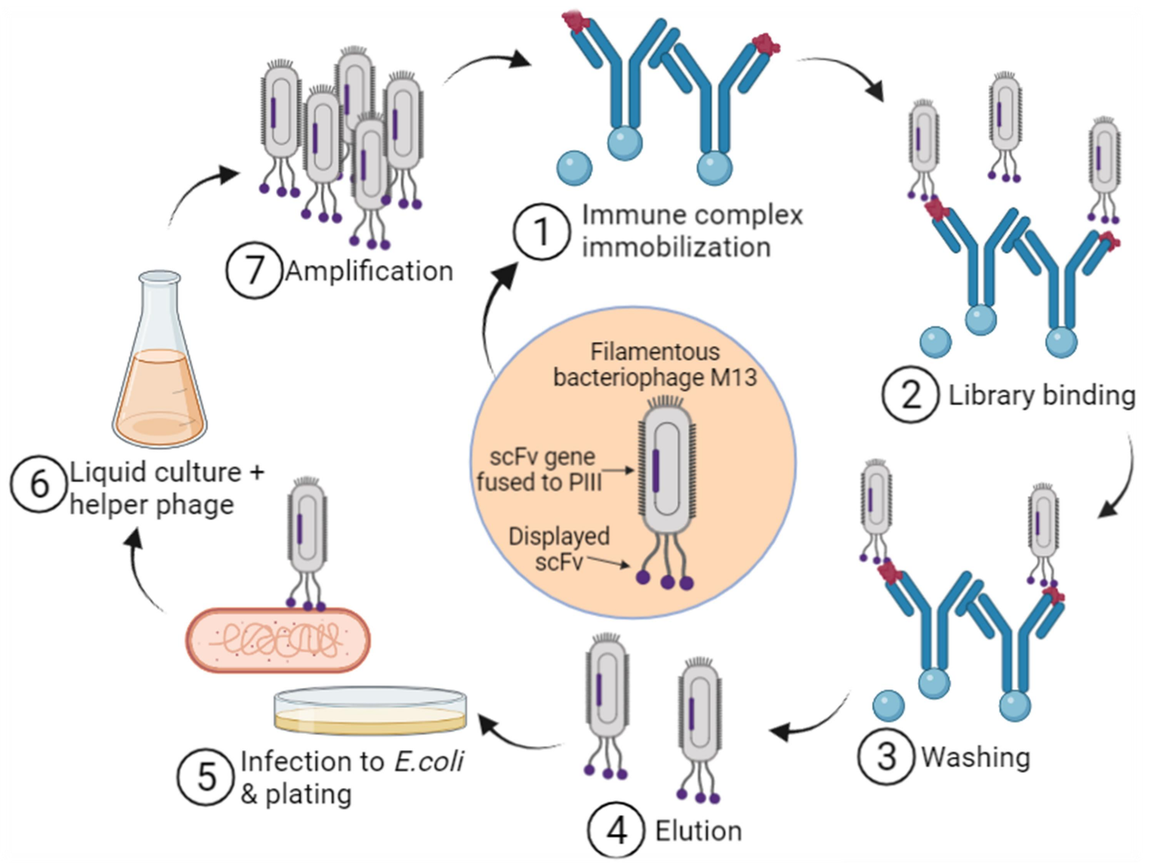


Aims

1. Develop anti-immune complex antibodies against anti-AFM1 monoclonal antibody bound to AFM1
2. Develop a sensitive non-competitive assay for AFM1 detection



Materials & Methods



Panning 3 rounds

- Synthetic scFvP library

Screening

- scFv is produced as a fusion protein with AP
- Individual binders are selected

Characterization

- Sequencing
- Cross-reactivity
- Analytical performance

Optimization of an assay

- Antibody concentrations
- Washing steps
- Assay buffer

Results

Panning

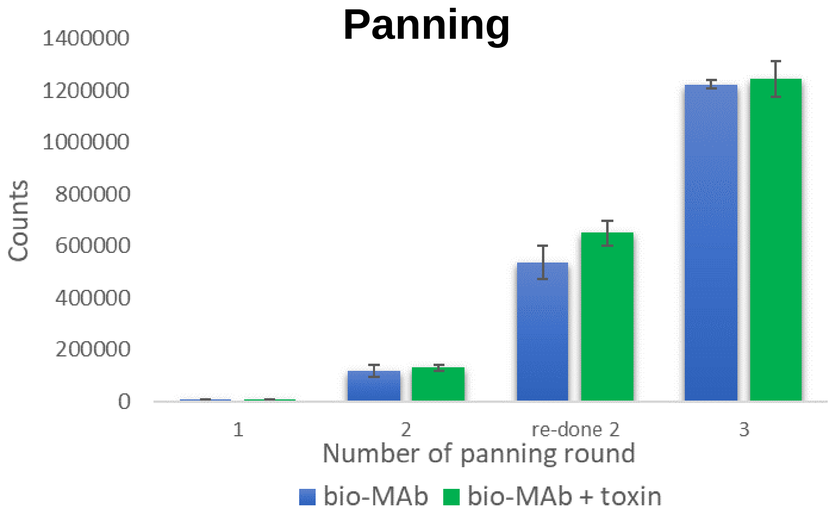


Figure 1. Immunoreactivity of phage libraries. Measured time-resolved fluorescence signals to binding of selected phage libraries against bio-anti-AFM1-mab and target immune complex.

Optimization of non-competitive immunoassay

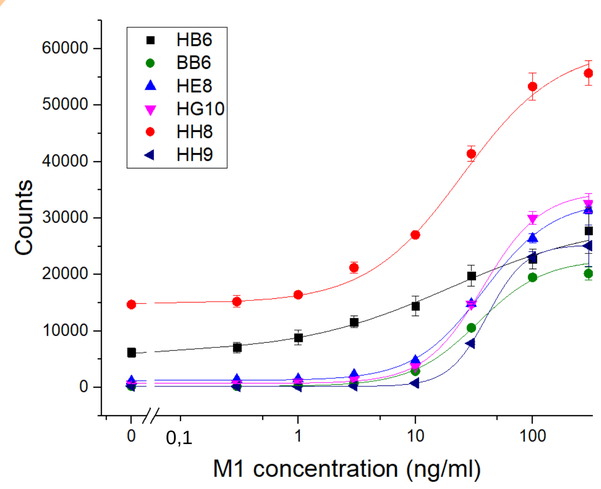


Figure 3. Optimized assay for antibody concentrations with six best immune complex scFv binders.

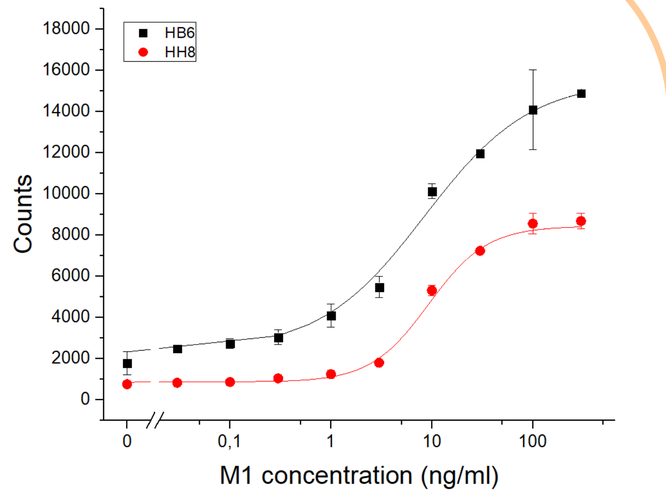


Figure 4. Optimized assay for washing steps and assay buffer with two best immune complex scFv binders. Assay exhibits EC_{50} values of 8.2 and 8.4 ng/ml for HB6 and HH8.

Screening

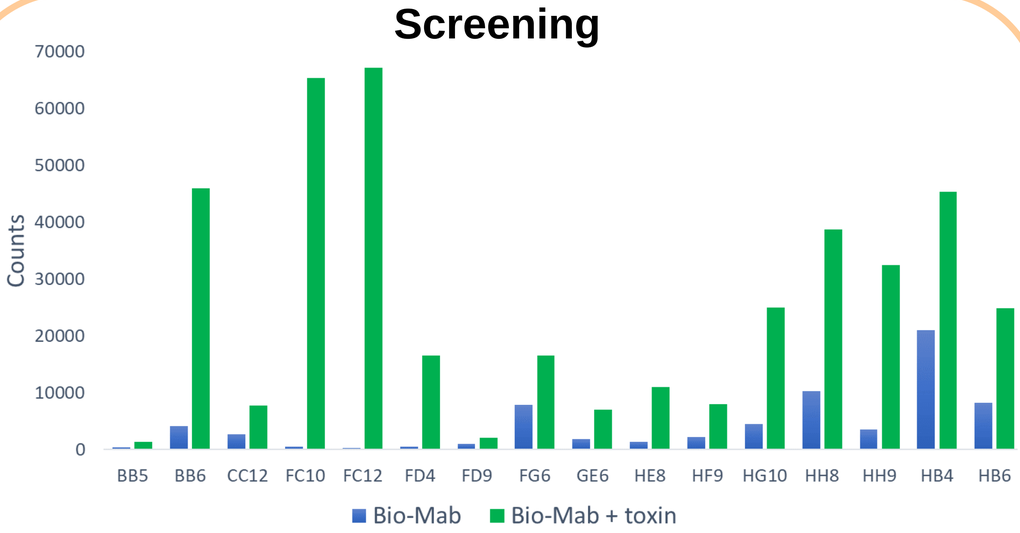


Figure 2. scFv-AP binders with a signal to background ratio > 2 of total 450 screened binders.

Actual spiked concentration (ng/ml)	Measured concentration (ng/ml)	Recovery (%)	RSD (%)
1	0.8	84.1	8.3
3	3.1	102.4	5.7
10	14.3	142.9	2.2
30	38.7	129.0	4.7
100	173.6	173.6	11.6

Table 1. Recovery test with milk samples using HB6 binder**.** The table shows the amount of AFM1 measured as a percentage recovery of the original spiked concentration.

Conclusions

Anti-immune complex antibody for AFM1 was successfully selected from synthetic antibody library and it was applied to the development of a non-competitive assay for AFM1 detection. The assay showed good analytical performance and exhibited acceptable recoveries in milk samples.