Electronic Supplementary Material

Colour-encoded lateral flow immunoassay for the simultaneous detection of aflatoxin B1 and type-B fumonisins in a single Test line

Fabio Di Nardo, Eugenio Alladio, Claudio Baggiani, Simone Cavalera, Cristina Giovannoli, Giulia Spano, Laura Anfossi*

Department of Chemistry, University of Turin. Via Giuria, 5, I-10125 Turin, Italy

*to whom correspondence should be addressed. Tel: +390116705219, fax: +390116705242. E-mail: laura.anfossi@unito.it

Preparation of the FMB1-BSA conjugate

To synthesise the FMB1–BSA conjugate, 6.5 mg of FMB1 were dissolved in 3 mL of MES-NaCl buffer (MES 0.1 M, NaCl 0.9 M at pH 4.7) and mixed with 3 mg of BSA dissolved in 0.6 mL of water. Then, 0.7 mL of a freshly prepared water solution of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydro-chloride (10 mg mL^{-1}) was added drop wise to the mixture and reacted for 2h at room temperature. By-products were removed by gel-filtration on a Sephadex G-25 cartridge (GE Healthcare Bio-Science, Sweden), by using phosphate buffer (sodium hydrogen phosphate-sodium dihydrogen phosphate 20 mM, pH 7.4) with 0.1 M NaCl added as the eluent. The concentration of the FMB1-BSA conjugate was determined by UV absorption at 280 nm.

Preparation of the AFB1-BSA conjugate

To synthesise the AFB1–BSA conjugate, 5.7 mg of the AFB1-oxime hapten were activated with equimolar amounts of N-hydroxysuccinimide and N,N'-diisopropylcarbodiimide (1:1:1) in anhydrous N,N-dimethylformamide for 2 hours (4°C) and then reacted overnight at room temperature with 5 mg of BSA dissolved in sodium bicarbonate 0.15 M at pH 8.3 and the pure conjugate was obtained from gel-filtration, as described for FMB1-BSA purification. AFB1-BSA concentration was determined through the Brilliant Blue Coomassie method. Conjugates were supplemented with 0.1% sodium azide and stored refrigerated.

Preparation of red-GNPs and GNPs seeds

'Red' GNPs with a SPR band at 525 nm and mean diameter of ~ 30 nm were synthesised as follow: tetrachloroauric acid was dissolved in 100 mL ultrapure water (0.01%, w/v) and the obtained solution was

brought to boil. Then, 1 mL of 1% w/v sodium citrate aqueous solution was added to the boiling solution while stirring vigorously. The solution was stirred under boiling for further 10 minutes, then cooled down to room temperature.

GNPs seeds with a SPR band at 518 nm (mean diameter ~ 10 nm) were synthesised according to the aforementioned approach, using a double amount of reducing agent.

Preparation of blue-GNPs

'Blue' GNPs were synthesised through a seeding growth approach, as previously described by Di Nardo et al. [17]. Blue-GNPs with a SPR band at 620 nm and hydrodynamic diameter of ca. 70-75 nm were prepared as follow: $1.9 * 10^{-8}$ mol of $HAuCl_4$ was mixed with $9.3 * 10^{-13}$ mol of GNPs seeds at room temperature (10 ml); $7.5 * 10^{-9}$ mol of sodium citrate, dissolved in 0.2 ml ultrapure water, was added to the mixture and stirred for 2 min in order to homogenize. Then, $3.0 * 10^{-5}$ mol of hydroquinone (1.5 ml) was rapidly added to the solution, under stirring. The solution was kept under stirring at room temperature for further 20 min and the obtained sol exhibits a blue colour, which is consistent with the SPR band observed. The sol was adjusted to the desired pH with carbonate buffer.

Preparation of red GNPs-labelled polyclonal antibodies

Red GNPs were adjusted to pH 7 with carbonate buffer (sodium carbonate-sodium bicarbonate 50 mM, pH 9.6) and 8 µg of anti-type B fumonisins antibody was added to 1 mL of red GNPs. After 30 min at 37 °C, 100 µl of borate buffer (20 mM pH 8.0) with BSA 1% w/v was added and the solution kept at 37 °C for 10 min. Then, the mixture was centrifuged for 10 min at 25 °C (18400 x g) and the pellet was washed twice by re-suspension in borate buffer with BSA 1% w/v, sucrose 2% w/v, Tween 20 0.25% v/v and sodium azide 0.02% w/v.

Preparation of blue GNPs-labelled anti-FMB1 antibodies

Blue GNPs were adjusted to pH 5 with carbonate buffer and 16 µg of anti-aflatoxin B1 antibody was added to 1 mL of blue GNPs. After 30 min at 37 °C, 100 µl of borate buffer (20 mM pH 8.0) with BSA 1% w/v was added and the solution kept at 37 °C for 10 min. Then, the mixture was centrifuged for 15 min at 4 °C (6800 x g) and the pellet was directly resuspended in borate buffer with BSA 1% w/v, sucrose 2% w/v, Tween 20 0.25% v/v and sodium azide 0.02% w/v.

Assessment of accuracy and the within- and between-day precision.

Five mixtures of AFB1 and FMB1 were prepared variously combining the two mycotoxins (Table S1) to simulate samples contaminated at cut-off levels and sample non-contaminated (mycotoxin level ½ cut-off or absent). Analysis was repeated twice on the same day and on three different days.

Table S1. Combinations of AFB1 and FMB1 added for accuracy and within- and between-day precision assessment.

Combination #	Aflatoxin B1 level	Fumonisin B1 level
1	-	cut-off
2	½ cut-off	cut-off
3	cut-off	-
4	cut-off	½ cut-off
5	cut-off	cut-off

Fig. S1. Mixture # 1 comprising 0 ng mL^{-1} AFB1 (-) and 50 ng mL^{-1} FMB1 (cut-off level). The red colour at the Test line indicates contamination due to the presence of FMB1 exceeding the cut-off level.

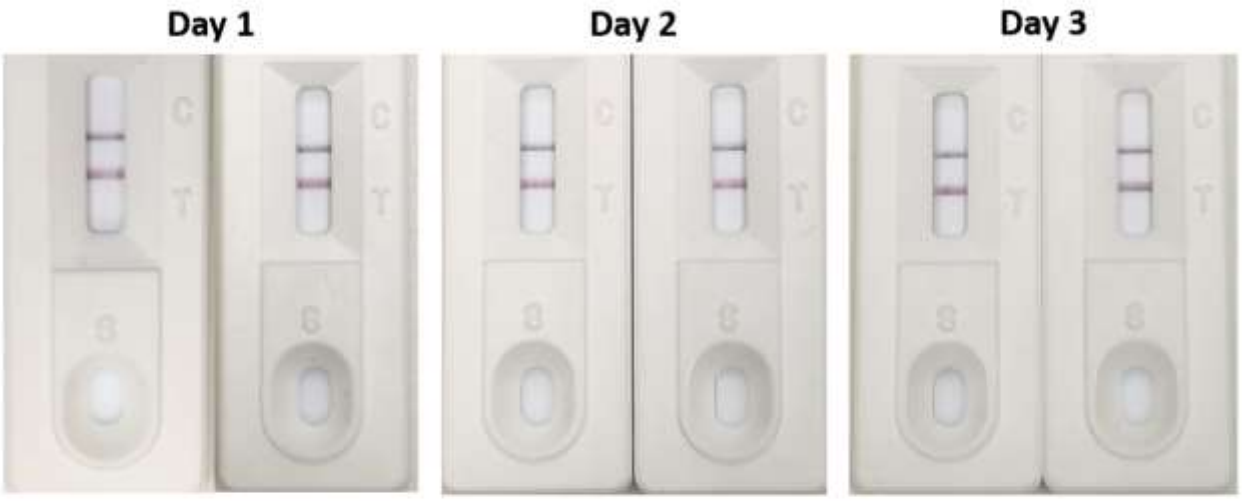

Fig. S2. Mixture #2 comprising 0.5 ng mL^{-1} AFB1 (1/2 cut-off) and 50 ng mL^{-1} FMB1 (cut-off). The red colour at the Test line indicates contamination due to the presence of FMB1 exceeding the cut-off level.

Fig. S3. Mixture #3 comprising 1 ng mL^{-1} AFB1 (cut-off) and 0 ng mL^{-1} FMB1 (-).The blue colour at the Test line indicates contamination due to the presence of AFB1 exceeding the cut-off level.

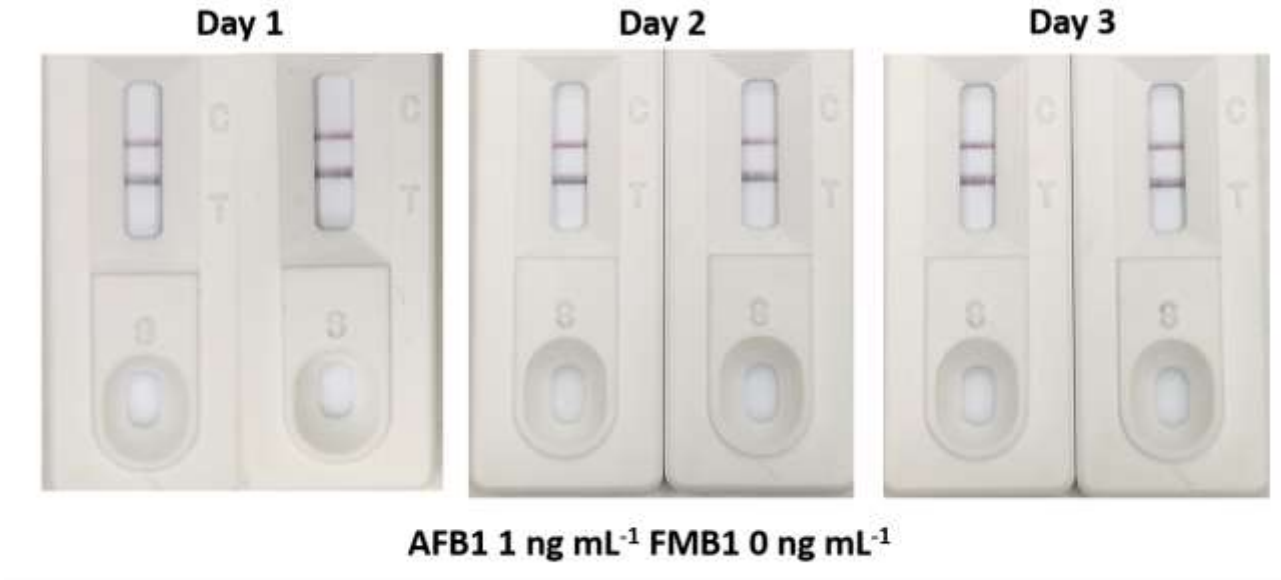

Fig. S4. Mixture #4 comprising 1 ng mL^{-1} AFB1 (cut-off) and 25 ng mL^{-1} FMB1 (1/2 cut-off). The blue colour at the Test line indicates contamination due to the presence of AFB1 exceeding the cut-off level.

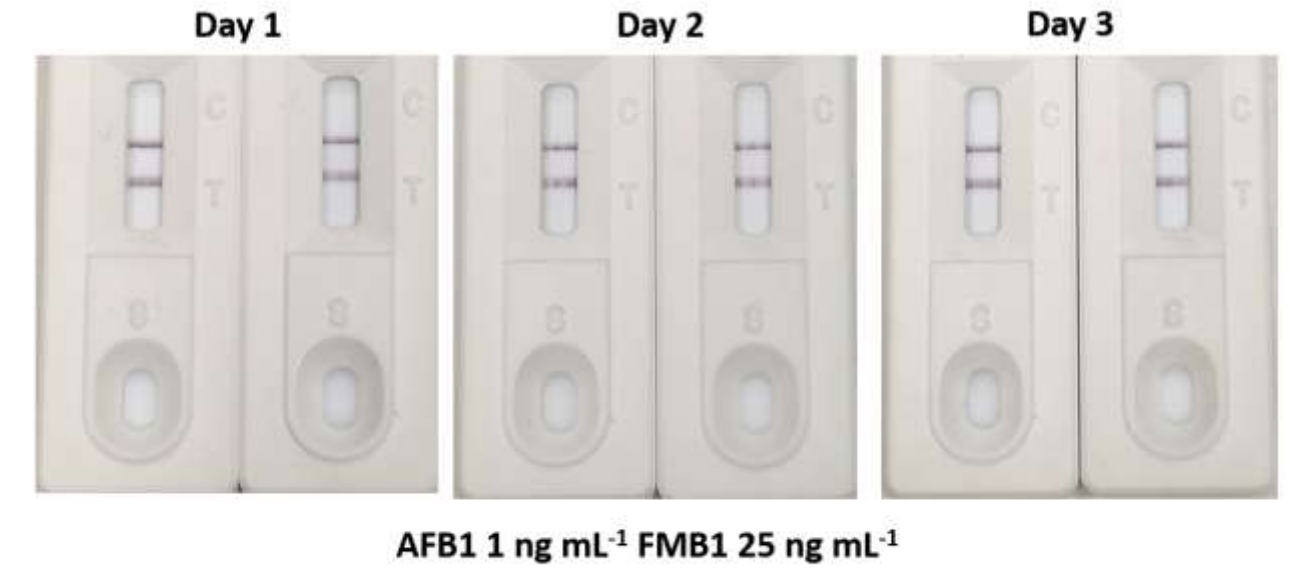

Fig. S5. Mixture #5 comprising 1 ng mL^{-1} AFB1 (cut-off) and 50 ng mL^{-1} FMB1 (cut-off). The pale purple colour at the Test line (compared to the Control line) indicates contamination due to the simultaneous presence of AFB1 and FMB1 exceeding the cut-off level.