Lab-on-a-Chip System for Detection of Mycotoxins in Animal Feed

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Introduction

Mycotoxins are health hazardous metabolites produced by fungi growing on foodstuffs or animal feed. Upon exposure they impact on the productivity of livestock including diary products. As the result of livestock feeding on contaminated commodities, mycotoxins carry over to the human food chain via meat or other animal products. This poses a high risk to human health as some of the most common mycotoxins are carcinogenic, genotoxic, or target the kidney or liver. It is now well established that acute mycotoxicoses (the diseases caused by mycotoxins) have been responsible for major epidemics in man and animals.

Since mycotoxins are robust, small molecules that are not easily destroyed by temperature treatments occurring during cooking, baking or freezing processes and even fermentation, it is necessary to avoid usage of contaminated food and feed upfront. However, the presence of mycotoxins is not detectable by visual screening due to the fact that high amounts of toxins may still exist even after removal or destruction of the generating organisms. Hence availability of sensitive, accurate and affordable tests for the detection and quantisation of mycotoxin in feeds is of great interest for the care of health.

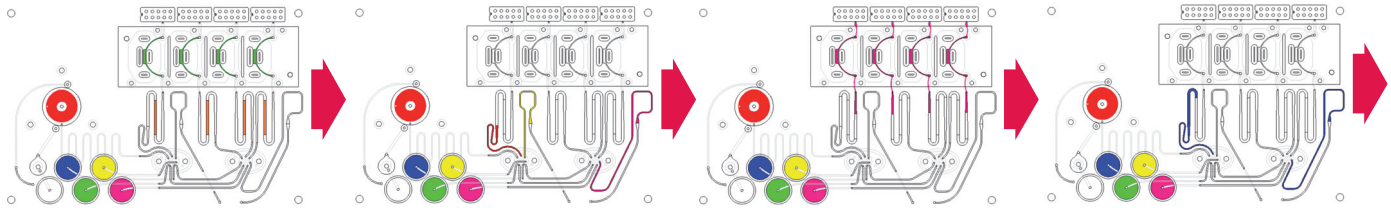
Fig. 1: *Disposable polymer chip with attached extraction unit inserted into the fully automated device incl. actuation and detection modules.*

Here, we introduce an automated **Lab-on-a-Chip system for reliable quantitative testing of mycotoxin contamination of animal feed** (cf. figure 1) developed in the framework of the EU-funded project microFLUID [1].

Employment of microfluidic analysis techniques facilitates miniaturisation, allowing for the use of small quantities of reagents to perform assays. This reduces costs and, avoids the handling of large amounts of dangerous toxins. The portability of the system enables in-field analysis and provides a significant improvement in the prevention and control of the contamination. The instrumental reading of the optical intensities ensures a reliable and quantitative interpretation of the result.

System Design and Results

The LOC system is based on a **disposable microfluidic polymer chip** (cf. Fig 1) equipped with an **optical immunoassay technique for detection**. Due to small channel dimensions, substantially reduced incubation times are achieved compared to these typically required in respective commercial assays carried out in 96-well plates. Furthermore, our fully automated system allows for **direct detection of mycotoxins in raw samples** [2,3]. In the extraction process recovery rates higher than 80 % have been found. After extraction, the analyte is automatically transferred to microfluidic channels where immunoassay-based fluorescence detection takes place. The concept of the implemented ELISA is illustrated in the following figures:

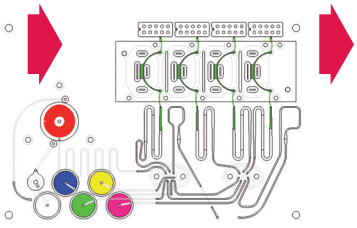


Step 1: Extraction of analyte *(here: Aflatoxin B1) in attached unit (large red cycle) or external. Lyophilised reagents (orange channels), surface immobilised antigens (green channels) are stored on-chip. Containers holding buffer or extracting agent (coloured circles) are attached to the chip.*

Step 2: Metering *of analyte (red), dilution buffer (yellow) and buffer (magenta) for the re-dissolving of lyophilised calibrators and/or conjugate (= antibody-HRP) in specifically designed metering loops. Subsequently, reagents pre-stored in channels are* ***re-dissolved*** *by bidirectional movements of fluid plugs.*

Step 4: Incubation of analyte/ conjugate mix and calibrator/ conjugate solutions *(magenta) in detection channels above immobilised antigens. Excess antibodies which are not bound to an antigen stemming from calibrator or analyte will bind to the antigens immobilised previously to the channel surface.*

Step 5: Metering of washing buffer *and subsequent* ***washing of detection channels.*** *Non-immobilised duplexes of conjugate and antigens are removed to waste containers behind the detection area.*



Step 6: Metering of substrate *and subsequent* ***incubation of substrate in detection channels.*** *During the incubation a time the substrate (here: Amplex Ultrared) is converted to its fluorescing form. Thus, the resulting fluorescence signal is invers to the mycotoxin concentration present in the analyte.*

Step 7: Read out *of fluorescence signal by a large area photodiode below detection channel.*

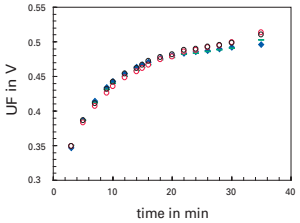


Fig 2: *Dynamic measurement of fluorescence signal U_F during aflatoxin B1 assay (different symbols for each channel).*

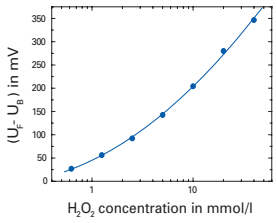


Fig 3: *Fluorescence signal U_F in Amplex Ultrared/peroxide assay (background signal U_B subtracted).*

The introduced mycotoxin detection system allows for automated direct **detection of mycotoxins at production sites by moderately trained personnel,** thereby providing a highly effective means to minimize mycotoxin contamination of livestock during the food production chain.

The principle design of the disposable microfluidic chip is **highly compatible with cost-effective, large-scale fabrication techniques** such as injection moulding. Most importantly, our LOC system can be readily equipped for simultaneous detection of all relevant mycotoxins in animal feed when initially implemented calibrators are exchanged by different mycotoxins.

References

[1] http://www.ifn.cnr.it/microfluid

[2] Further development of device covered by patent application DE102008042581.8-41

[3] M. Jakubowski, diploma thesis, Institut für Mikrotechnik Mainz, University of Applied Sciences Bingen, 2009

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