A rapid immunodiagnostic strip test for the quantitative determination of DON in wheat

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Introduction

Mycotoxins are secondary metabolites produced by fungal species which affect crops in the fields or after harvest and thus form a potential threat to human and animal health since they may be carcinogenic, genotoxic and immunsuppressive. The most common mycotoxins are aflatoxins (*Aspergillus spp.*), ochratoxins, patulin (both *Aspergillus* spp. and *Penicillum spp.*) and fusarium toxins including trichothecenes and zearalenone (both *Fusarium* spp.). Deoxynivalenol (DON) belongs to the type-B trichothecenes growing on grains such as wheat, maize, barley and oat.

The maximum level of DON in unprocessed cereals other than durum wheat, oat, and maize is set to 1250 µg/kg and in unprocessed durum wheat, oat, and maize to 1750 µg/kg (EC 1881/2006). The U.S. advisory level for DON in finished wheat products is set to 1000 µg/kg (FDA 1993). Cost effective and rapid on-site mycotoxin testing methods have increasingly gained importance in the last few years. A lateral flow device (LFD) for the quantitative determination of DON was developed. The presented one-step rapid immunochromatographic strip tests allow quantitative sample screening within a few minutes.

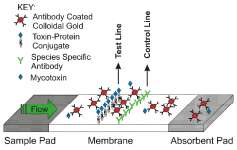
Experimental

Strip production:

The strip test is composed of different overlapping membranes. A nitrocellulose membrane, onto which the reagents for test and control line were immobilized by using a contact tip dispenser (BioDot, Irvine, CA), and a wick membrane for absorbing excess liquid were used. The test line was sprayed with DON mycotoxin coupled to a carrier protein and the control line with an anti-species specific antibody used for verifying the correct test performance.

Sample preparation:

- § Grind a representative wheat sample
- § Extract 10 g of sample 1:8 (w/v) with distilled water by shaking for 3 min
- § Allow extract to settle down for 10 min

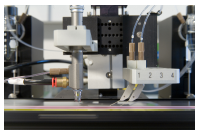


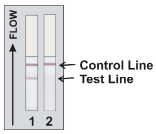
Test procedure:

50 µL of wheat extract are added to a microtiter well together with ready-to-use gold conjugate mix. The strip is inserted into the well. The result is read out after 3 minutes by using a handheld reader. The test is valid when the control line is visible.

Strip production:

Free binding sites of the membrane were blocked with a protein for preventing background signals and to ensure a homogenous flow. A conjugate made of DON-specific monoclonal antibody bound to colloidal gold with a particle size of about 40 nm was used as signal reagent. The type of membrane, the coupling ratio of the gold conjugate, and the concentration of the mycotoxin conjugate mainly affected the signal intensity of the test line and were optimized.





Results and Discussion

Strip test optimization:

Several optimizations were carried out for the production of the quantitative DON strip:

- § Finding the proper monoclonal antibody to gold colloid ratio (see Figure 1)
- § Comparing different protein-toxin conjugates such as bovine serum albumin, ovalbumin, and conalbumin A (see Figure 2)

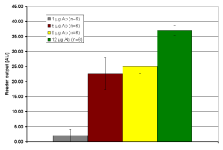


Figure 1: Effect of antibody coupling ratio on colloidal gold on the signal intensity.

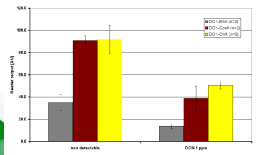
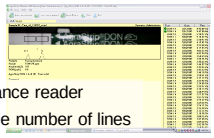


Figure 2: Changes in signal intensity with the protein type used in mycotoxin-protein conjugate

Strip Reader:



- Photometric reflectance reader
- Screening of variable number of lines
- Data files stored automatically with picture of strip

Matrix-matched calibration:

A highly contaminated wheat sample was extracted and the extract diluted in a concentration range from 25 to 1250 ppb with blank extract and measured by a handheld photometric reader. A calibration curve was performed as e.g. shown on Figure 3 which was used for the determination of the DON concentration range in unknown samples.

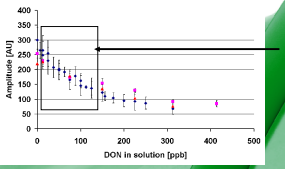


Figure 3: Matrix-matched calibration of DON strip test with naturally contaminated wheat extracts blended with blank extract (1:8 extraction with dest. water). (n=3).

The linear range selected for method development is shown on Figure 3 (see square).

Measurement of naturally contaminated samples:

Naturally contaminated wheat samples with a concentration range from 50 ppb to 1300 µg/kg have been measured. An LC-MS-MS method as described by Sulyok et al. (*Rapid Commun. Mass Spectrom*. 20, 2649-2659, 2006) was used as reference method for the characterization of the naturally contaminated samples.

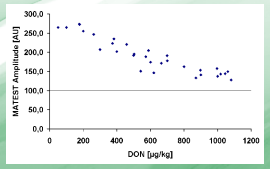


Figure 4: Measured naturally contaminated wheat samples. 1:8 sample extraction with dest. water. DON strip test results vs. reference LC-MS-MS method.

Measurement Nr.	AgraStrip DON [µg/kg]	Measurement Nr.	AgraStrip DON [µg/kg]
1	996	15	1163
2	1295	16	1110
3	1270	17	1110
4	1295	18	1163
5	1118	19	1163
6	1330	20	1097
7	1097	21	1126
8	996	22	1034
9	890	23	1057
10	968	24	730
11	1072	25	886
12	1110	26	1102
13	1034	27	1209
14	1148	28	1019
Mean			1068
STD			136
RSD [%]			12

Table 1: Measurement of strip-to-strip reproducibility (n=28). Wheat sample with DON concentration of 997 µg/kg.

Furthermore, the precision of the DON strip has been studied by running 28 strip tests on a wheat sample naturally contaminated with 997 µg/kg DON as shown on Table 1. Results were in agreement with the reference measurements obtained for DON. The RSD of the test was 12%.

Conclusion and Outlook

Rapid strip tests for the quantitative determination of deoxynivalenol in wheat and wheat flour have been developed. With no sample clean up required, an extraction time of 3 minutes, and a read out time of 3 minutes, the overall test time was reduced to 8 minutes.

The quantitative strip test can be used for the determination of DON in the concentration range of 0 to 1300 ppb with a photometric reflectance reader. The strip test will be tuned for further commodities such as maize, dried destillers grain, oat and barley.

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