# Multiple sample screening with aqueous aflatoxin extraction – New RIDASCREEN®FAST Aflatoxin ECO ELISA

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## Introduction

Due to their highly carcinogenic and immunosuppressive mode of action, aflatoxins are a threat to human and animal health. Hence, testing for aflatoxin contamination is crucial. Recognizing the difficulty of disposing sample extracts containing organic solvents, R-Biopharm AG has developed a new GIPSA approved (FGIS 2017-098) test – **RIDASCREEN®FAST Aflatoxin ECO ELISA (Art. No. R5201)** which is completely built on a water-based extraction method, making the disposal of sample extracts with non-detectable aflatoxins hassle-free.

## Aim of the study

To combine the possibility to cover the complete GIPSA conformance range for aflatoxin (5 - 300 µg/kg) in one test run with a multiple sample screening method, we developed the RIDASCREEN®FAST Aflatoxin ECO ELISA test system for total aflatoxin (B1, B2, G1, G2). To additionally avoid the use of organic solvents, we developed a new, efficient and non-toxic aqueous extraction method. This minimizes the risks associated with the usage of organic solvents like methanol and also reduces the disposal costs for non-contaminated sample extracts. Additionally, the incubation time was set to a total of 8 min in order to develop a rapid test system.

## Material and methods

One of the most important commodities frequently contaminated with aflatoxins is corn. To demonstrate the conformance of ELISA testing results with HPLC results, we tested ten naturally contaminated Trilogy® corn reference materials with two different batches of RIDASCREEN®FAST Aflatoxin ECO. The contamination levels of the reference materials covered the complete detection range of 5 - 300 µg/kg. The Trilogy® reference material contamination values generated by HPLC and mentioned on their certificates were set as target values.
To further prove the quality of the system with an external confirmation, the test system was submitted to GIPSA for performance testing.

## Results

We were able to show very good recoveries with naturally contaminated corn samples. The ten tested reference materials showed a mean recovery of 97 % for batch 1 and of 101 % for batch 2 (see Table 1). Total standard deviations of recovery values per batch were as low as 0.082 and 0.081, respectively. To test for repeatability, identical samples were extracted on 3 different days and tested n = 8 times on the same day.

The results of a tested non-detect sample analyzed with 2 different batches of RIDASCREEN®FAST Aflatoxin ECO showed very low total coefficients of variation (CV) of 2.1 - 3.8 % per day (see Table 2). The identical experiment performed with a Trilogy® naturally contaminated reference material showed CVs ≤ 8.9 %. RIDASCREEN®FAST Aflatoxin ECO has been approved by GIPSA (FGIS 2017-098).

**Table 1:** Recovery was tested by comparing the HPLC-results of Trilogy® reference material mentioned on their certificates to the concentration (c) values of the identical samples analyzed with RIDASCREEN®FAST Aflatoxin ECO. Data was generated with 2 different batches of the ELISA test system.

| Corn | Total aflatoxin (Trilogy® HPLC) | ELISA results (RIDASCREEN®FAST Aflatoxin ECO) | | | |
|---|---|---|---|---|---|
| | | Batch 1 | | Batch 2 | |
| | [µg/kg] | c [µg/kg] | Recovery | c [µg/kg] | Recovery |
| AC-287 | 5.3 | 5.6 | 105 % | 5.5 | 103 % |
| AC-274 | 7.3 | 6.6 | 90 % | 7.0 | 96 % |
| AC-289 | 9.6 | 9.1 | 95 % | 8.6 | 89 % |
| AC-294 | 14.6 | 15.9 | 109 % | 16.6 | 113 % |
| AC-286 | 20.2 | 19.6 | 97 % | 20.1 | 99 % |
| AC-288 | 21.7 | 18.7 | 86 % | 23.0 | 106 % |
| AC-290RB | 32.3 | 33.8 | 105 % | 34.2 | 106 % |
| AC-277 | 50.8 | 52.3 | 103 % | 47.9 | 94 % |
| AC-279 | 98.7 | 86.7 | 88 % | 109.5 | 111 % |
| AC-2219 | 282.0 | 252.6 | 90 % | 262.3 | 93 % |
| **Mean recovery** | | | **97 %** | | **101 %** |
| **Standard deviation** | | | **0.082** | | **0.081** |

**Table 2:** Repeatability data was generated with a non-detect sample and a contaminated Trilogy® reference material. For this, the identical sample was extracted on 3 different days and tested n = 8 times in one run on the day of extraction. Mean $B/B_0$ values and mean CVs as well as total CVs are shown for the non-detect samples. Mean concentrations (c) in µg/kg, mean CVs and total CVs are shown for the Trilogy® reference material.

| | | Batch 1 | | Batch 2 | | |
|---|---|---|---|---|---|---|
| **Corn** | | **Mean $B/B_0$** | **Mean CV [%]** | **Mean $B/B_0$** | **Mean CV [%]** | **Total CV [%]** |
| Non-detect | Day 1 | 97.0 | 1.6 | 93.9 | 2.5 | **2.6** |
| | Day 2 | 98.7 | 2.3 | 93.2 | 2.7 | **3.8** |
| | Day 3 | 95.1 | 2.1 | 95.9 | 2.0 | **2.1** |
| **Trilogy® corn reference material** | | **Mean c [µg/kg]** | **Mean CV [%]** | **Mean c [µg/kg]** | **Mean CV [%]** | **Total CV [%]** |
| AC-2215 21 µg/kg | Day 1 | 21.9 | 8.5 | 20.3 | 6.9 | **8.4** |
| | Day 2 | 19.0 | 3.5 | 21.6 | 5.9 | **8.2** |
| | Day 3 | 18.7 | 7.9 | 20.3 | 8.2 | **8.9** |

## Conclusion

RIDASCREEN®FAST Aflatoxin ECO is an optimal tool for the fast screening of corn samples by ELISA. The complete GIPSA conformance range is covered by the wide detection range of the test system without the need to perform additional dilutions. The aqueous buffer extraction is efficient and cost-saving by making the purchase of organic solvents redundant and by simplifying the disposal of sample extracts. Together with the very short incubation time of 8 minutes, RIDASCREEN®FAST Aflatoxin ECO is an optimal solution for rapid testing needs according to GIPSA regulations that allow for the testing of multiple samples in one test run.

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