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Cross-Reactivity of Antibodies in Some Commercial Deoxynivalenol Test Kits against Some Fusariotoxins

Emmanuel K. Tangni,* Jean-Claude Motte, Alfons Callebaut, and Luc Pussemier

Veterinary and Agrochemical Research Centre (CODA-CERVA), Operational Directorate of Chemical Safety of the Food Chain, Unit of Toxins and Natural Components, Leuvensesteenweg 17, 3080 Tervuren, Belgium

Cross-reactivity of antibodies in AGRAQUANT, DON EIA, VERATOX, ROSA LF-DONQ, and MYCONTROLDON designed for deoxynivalenol (DON) determination in food and feedstuffs was evaluated against nivalenol, 3-acetylDON, 15-acetylDON, de-epoxy metabolite 1 of DON, DON-3β-glucoside, T2-toxin, HT2-toxin, fusarenone X, diacetoxyscirpenol, verrucarol, and zearalenone. Cross-reactivity measurements were run in water using the 50% reduction of absorbance of the blank for ELISA kits or through direct DON determination upon using the standards of mycotoxins via ROSA LF-DONQ or MYCONTROLDON. For the tested toxin concentrations, all DON kits have low cross-reactivity toward diacetoxyscirpenol, T2-toxin, HT2-toxin, verrucarol, and zearalenone and moderate cross-reactivity toward 15-AcetylDON and fusarenone X. AGRAQUANT, DON EIA, and VERATOX kits showed high cross-reactivity in various ranking orders against DON-3-Glc, DOM-1, and 3AcDON. DON EIA showed also high cross-reactivity against nivalenol and fusarenone X. These mycotoxins could coexist in food or feedstuffs, and analytical results can be wrongly interpreted. Cross-reactivity does not allow checking the compliance with the legal norms, but it does allow an overall risk assessment for the consumers. Updating regularly the cross-reactivity evaluation of the produced batches is recommended for 3-acetylDON, nivalenol, DON-3-Glc, de-epoxy metabolite 1, and fusarenone X.

KEYWORDS: Mycotoxins; deoxynivalenol; cereals; fast test; cross-reactivity

INTRODUCTION

Fusariotoxins are toxic secondary metabolites produced under field conditions by many species of *Fusarium* in various cereals, foods, and feedstuffs. Among them, the trichothecenes are a family of several related cyclic sesquiterpenoids, which are divided into four groups (types A−D) according to their characteristic functional groups (*1*). Type A and B trichothecenes are the most common. Type A is represented by HT-2 toxin and T-2 toxin. Chronic exposure to T2-toxin reduces feed intake and body weight gain in pigs and induces mucocutaneous lesions in the gastrointestinal tract. T2-toxin is an inhibitor of protein synthesis, hematotoxic and immunotoxic with deleterious effects on the cell-mediated and humoral acquired responses. Type B is most frequently represented by deoxynivalenol (DON), monoacetylated derivatives (3-acetylDON and 15-acetylDON), nivalenol, and fusarenone X. Nivalenol is a mycotoxin often co-occurring with diacetoxyscirpenol in cereals depending on geographical area or meteorological conditions of the year. Type C and D trichothecenes are characterized by a second epoxide (C-7,8 or C-9,10) or an ester-linked macrocycle (C-4,16), respectively, and are not associated with *Fusarium* head blight. The toxic effects of trichothecenes include gastrointestinal effects such as vomiting, diarrhea, and bowel inflammation. Anemia, leukopenia, skin irritation, feed refusal, and abortion are also common. The trichothecenes as a group are immunosuppressive. Susceptibility varies considerably among species, but pigs are generally recognized as the most sensitive animal species. Other species, including rabbits, horses, cats, and dogs, seem to have a higher tolerance toward DON than pigs (*1*, *2*).

Species of *Fusarium* strains typically grow under moderate climate conditions able to infect a variety of cereal crops before harvest. They are potential fusariotoxin concerns for European cereals and cereal products (*3*). DON may significantly co-occur with zearalenone mainly produced by *Fusarium graminearum* and *Fusarium culmorum* or with fumonisins (especially fumonisin B1) mainly produced by *Fusarium verticilloïdes*. Zearalenone has an estrogenic action and is significantly toxic to the reproductive system of animals (*4*). In addition to the native mycotoxins and as part of their metabolism, plants are capable of transforming mycotoxins into conjugated forms. So far, natural occurrences of zearalenone-4-glucopyranosides and DON-3β-glucoside (DON-3-Glc) have been reported (*5*, *6*). They naturally occurred in *Fusarium*-contaminated cereals (e.g., of 23 wheat samples originating from fields in Austria, Germany, and Slovakia and 54 maize samples from Austrian fields, DON and DON-3-Glc were detected in all 77 field samples). DON was found at levels ranging from 42 to 4130 μg/kg, whereas DON-3-Glc, found in all cereal samples, ranged from 10 to 1070 μg/kg (*7*). Via the raw agricultural commodities, native and masked mycotoxins can

*Author to whom correspondence should be addressed (e-mail emtan@var.fgov.be; phone 0032 2 769 2243).

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contaminate many different foods such as cornflakes, meal flour, malt, and beers (*8*−*10*). In animals, following absorption, DON is rapidly metabolized by de-epoxidation and glucuronidation, yielding less toxic metabolite (de-epoxy metabolite 1). Chemically, it belongs to the trichothecenes and is not a naturally occurring contaminant in foods and feedstuffs. It has nearly the same polarity as the parent substance DON (*11*, *12*).

For the effectiveness of the mycotoxin control, immunoassay techniques using polyclonal or monoclonal antibodies have been developed to detect DON or other mycotoxins in grains, foods, feeds, and animal products (*13*−*16*). They provide a simple and economical alternative to instrumental methods for mycotoxin analysis. Robust indirect and direct competitive ELISAs, commercially available in various formats, are commonly used for the most important *Fusarium* mycotoxins (e.g., DON, zearalenone, T2-toxin, and fumonisins). Using one commercially available ELISA kit for the examination of 313 beer samples collected from the European retail markets, it was found that about 87% are DON contaminated (*17*). Furthermore, checking the contribution of DON-3-Glc to ELISA DON results, the occurrence of DON-3-Glc in beer at levels exceeding that of free DON in some samples has been observed (*10*). Within a group of closely related mycotoxins, structures are sometimes very similar and antibody-based techniques may be prone to cross-reactivity. Indeed, ELISAs for DON often show high cross-reactivity for 3-acetylDON, 15-acetylDON, or DON-3-Glc, making an accurate quantification of DON by immunological assays difficult (*15*, *18*, *19*). This may also explain the high recoveries exceeding 100% that are typically obtained by DON ELISA kits when the results of naturally contaminated wheat analysis generated by an accurate LC-MS/MS method are taken as reference (*14*). Some methods therefore entail acetylation of the toxins in the cereal extract before the DON assay and result in the determination of the sum of DON and its acetylated derivatives (*20*).

In addition to ELISA kits, lateral flow devices (LFDs) are user-friendly formats requiring a very short time for results. Fluorescent polarization immunoassays (FPIA), similar to immunosensors, are based on the competition between a mycotoxin and its fluorescent tagged tracer for a mycotoxin-specific antibody. In the presence of free toxin, the tracer binds less to the antibodies and the polarization signal is decreased. These assays are extremely rapid and easy to perform because they do not require washing steps. Additionally, they are automatable, and commercially available systems allow a high throughput (*15*, *16*).

If immunoassays offer a number of advantages in food contaminant analysis over the conventional methods such as liquid or gas chromatography-based methods, cross-reactivity is one of the series of limitations that should be pointed out as a drawback in using immunoassay kits (*16*, *21*). Extremely good antibodies are required for easy-to-use assays. Knowledge of assay specificity (cross-reactivity) is critical for correct data interpretation (*22*, *23*). Because cross-reactivity of sample components present in aqueous matrix extract to DON antibodies may differ depending on kit suppliers, the objective of the present study was to evaluate the cross-reactivity of antibodies used in some commercial kits devoted to DON determination in cereal and cereal products against some fusariotoxins.

MATERIALS AND METHODS

DON Kits. Three commercial ELISAs, AGRAQUANT (Romerlabs, Tulln, Austria), DON EIA (EuroProxima, Arhnem, The Netherlands), and Veratox for DON 5/5, Europe Only (Neogen Corp., Lansing, MI), one LFD, ROSA LF-DONQ (CHARM, Lawrence, MA), and one FPIA, Mycontrol DON (AOKIN AG, Berlin, Germany), were tested. A proprietary LFD reader of CHARM and FPIA spectrophotometer of AOKIN were used. All of these kits are designed for quantitative deoxynivalenol determination in cereal and cereal products (wheat, barley, corn, oats, malted barley, rice, wheat flour, wheat midds).

Note that all of the test procedures and the reagents contained in the kits are used as recommended by the providers. Prior to the practical exercises, demonstration trials were provided at CODA-CERVA by the manufacturers.

Mycotoxins and Reagents. Pure crystalline fusariotoxin standards of deoxynivalenol, nivalenol, fusarenone X, verrucarol, zearalenone, T2-toxin, and HT2-toxin were purchased from Sigma Chemical Co. (St. Louis, MO), whereas certified standard solutions of de-epoxy metabolite 1, DON-3-Glc, 3-acetylDON, 15-acetylDON, and diacetoxyscirpenol were obtained from Biopure (Tulln, Austria). All were dissolved in acetonitrile, stored at −20 °C in amber glass vials, and brought to room temperature before use. The concentrations were measured by spectrophotometer and showed good stabilities in acetronitrile, ethanol, or methanol during storage as checked during the study by HPLC-DAD measurements (coefficient of variation, CV, < 10%).

Acetonitrile, methanol, and ethanol were purchased from Biosolve (Valkenswaard, The Netherlands). They were of HPLC grade. Water of HPLC-quality was obtained using a twin system composed by Elix3 and Milli-Q systems, both from Millipore (Molsheim, France).

Study Design. *ELISA Microwell Devices*. The stock solutions of DON, nivalenol, 3-acetylDON, 15-acetylDON, de-epoxy metabolite 1, DON-3-Glc, diacetoxyscirpenol, fusarenone X, T2-toxin, HT2-toxin, verrucarol, and zearalenone were diluted in water (maximum ratio of solvent. to water < 1:100). Eight concentrations (0, 5, 10, 25, 40, 50, 75, and 100 ng/mL in standard solution) were assayed in duplicate within the same day in two different microwell plates. The assay procedure was strictly followed according to the instructions provided by the manufacturer. Optical densities (*B*) were recorded at 450 nm (for DON EIA, AGRAQUANT) and 650 nm (for VERATOX) using the microplate reader (Bio-Rad, Hercules, CA).

Provided (internal) and nonprovided (external) DON standards were tested for dilution linearity by linear regression analyses between $\log_{10}(B/B_0)$ and $\log_{10}$[DON concentrations].

LFD and FPIA Test Kits. As the ROSA LF-DONQ reader of CHARM or MYCONTROLDON devices of AOKIN generate directly readings in concentration (μg/kg of cereal), a different approach was used. Calibration curves were constructed with serial dilution of the toxins. Using the working solution (= 200 ng/mL), six dilutions (75, 50, 37.5, 25, 10, 5%) and the blank solvent (water) were directly assayed according to the analytical procedure recommended by CHARM or AOKIN.

Before this experiment, the dilution linearity was tested using the working standard (= 200 ng/mL) as previously described (*24*) with acceptable linearity ranging between −10 and +10% of the normalized means and CV < 10% for the replicates.

Data Handling and Calculation for Specificity. *ELISA Devices*. Dose−response curves were constructed by plotting the theoretical concentration ($\log_{10}[C, \mu g/kg]$) against the relative absorbance ($\log_{10}[B/B_0]$). From this calibration curve the concentration (IC_{50}) at which 50% extinction occurs is calculated. In a typical log versus $\log_{it}$ curve of an ELISA kit, this corresponds to the intercept. The relative cross-reactivity in perent can be calculated as

$$\text{cross-reactivity}_{\text{3-acetylDON}}\ (\%) = \text{IC}_{50[\text{DON}]}/\text{IC}_{50[\text{3-acetylDON}]} \times 100 \quad (1)$$

where $IC_{50[DON]}$ is the concentration of DON required for a 50% signal reduction of the zero standard and $IC_{50[3\text{-acetylDON}]}$ is the concentration of 3-acetylDON needed for a 50% signal reduction of the zero standard.

Note that duplicate assays were performed and that average relative absorbance (B/B_0) values are displayed in the graphs.

LFD and FPIA Devices. As the ROSA LF-DONQ and MYCONTROLDON reader generates directly readings in concentration (μg/kg of cereal), the cross-reactivity was calculated by comparing the slopes of correlation analyses

$$\text{cross-reactivity}_{\text{3-acetylDON}}\ (\%) = \text{slope}_{(\text{3-acetylDON})}/\text{slope}_{(\text{DON})} \times 100 \quad (2)$$

where $slope_{(DON)}$ is the slope of correlation analysis between the equivalent of DON contents in cereal and the responses assumed as DON contents

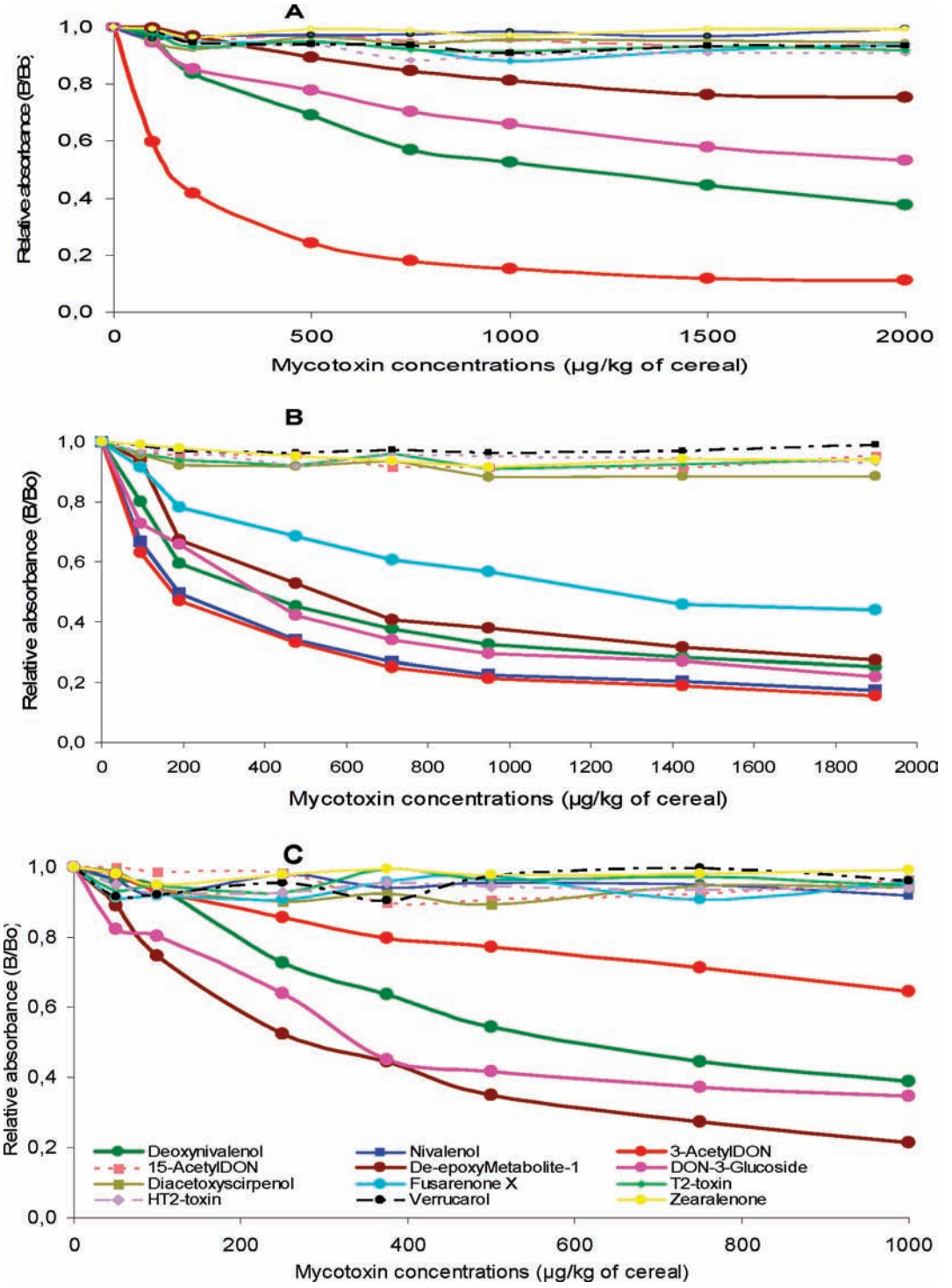

Figure 1. Standard curves (mean of duplicate assays) of deoxynivalenol (green), nivalenol (blue), 3AcetylDON (red), 15AcetylDON (coral), de-epoxy metabolite 1 (dark red), DON-3-Glc (pink), diacetoxyscirpenol (gray-green), fusarenone X (turquoise), T2-toxin (bright green), HT2-toxin (lavender), verrucarol (black), and zearalenone (yellow) as measured via AGRAQUANT (**A**), DON EIA (**B**), and VERATOX (**C**).

and $\text{slope}_{(3\text{-acetylDON})}$ is the slope of correlation analysis between the equivalent of 3-acetylDON contents in cereal and the responses assumed as DON contents.

Note that duplicate assays were also performed and averaged in the graphs. The Spearman correlation was used to study the consistency of the relationship between the equivalent of the tested mycotoxin concentrations (μg/kg of cereal) against the responses assumed as DON contents via ROSA LF-DONQ or MYCONTROLDON. The data were analyzed using the software PASW Statistics 18 (release 18.0.0 2009). Statement of statistical significance was based on a probability of $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of ELISA Antibodies against Some Fusariotoxins. The tested fusariotoxins (nivalenol, 3-acetylDON, 15-acetylDON, de-epoxy metabolite 1, DON-3-Glc, diacetoxyscirpenol, fusarenone X, T2-toxin, HT2-toxin, verrucarol, and zearalenone) diversely reacted against the antibodies in AGRAQUANT, DON EIA, and VERATOX (**Figure 1**).

The data were log-transformed and resulted in the IC_{50} values. As for deoxynivalenol, the IC_{50} values were 1097 ± 21, 405 ± 12, and 624 ± 56 μg/kg of cereal for AGRAQUANT, DON EIA, and

Table 1. Tested Fusariotoxins, Concentrations Required To Cause 50% Inhibition of Blank Signal (IC_{50}), and Cross-Reactivity of the Antibody Used in AGRAQUANT, DON EIA, and VERATOX

kit	DON	3-acetylDON	de-epoxy metabolite 1	DON-3-Glc	15-acetylDON	nivalenol	fusarenone X	diacetoxy scirpenol	T2-toxin	HT2-toxin	verrucarol	zearalenone
AGRAQUANT (ROMERLABS)												
mean IC_{50} (μg/kg)	1097	143	3109	2117	74340	NR[a]	228934	NR	NR	51109	NR	NR
SD (μg/kg)	21	16	35	84	28009	NR	174268	NR	NR	1753	NR	NR
CR (%)	100	770	35	52	2	0	0.7	0	0	2	0	0
SD_{CR} (%)	2	83	1	2	1	NR	0.5	NR	NR	0.1	NR	NR
DON EIA (EUROPROXIMA)												
mean IC_{50} (μg/kg)	405	176	484	352	NR	205	1272	NR	NR	NR	NR	NR
SD (μg/kg)	12	1.0	10	18	NR	12	76	NR	NR	NR	NR	NR
CR (%)	100	230	84	115	0	198	32	0	0	0	0	0
SD_{CR} (%)	3	1	2	6	NR	11	2	NR	NR	NR	NR	NR
VERATOX for DON 5/5 (NEOGEN)												
mean IC_{50} (μg/kg)	624	1567	296	399	NR	NR	NR	NR	NR	NR	NR	NR
SD (μg/kg)	56	147	23	4	NR	NR	NR	NR	NR	NR	NR	NR
CR (%)	100	40	212	157	0	0	0	0	0	0	0	0
SD_{CR} (%)	9	4	16	2	NR	NR	NR	NR	NR	NR	NR	NR

[a] NR indicates no cross-reaction, no statistical significant correlation between $\log_{10}$(DON concentrations) against values of $\log_{10}(B/B_0)$, no computation of the DON concentration needed for 50% reduction of absorbance of the zero standard.

VERATOX kits, respectively (**Table 1**). The IC_{50} values of all the tested toxins were compared with the DON IC_{50} values (= 100%) and resulted in the cross-reactivity percents for the monoclonal antibodies used in AGRAQUANT, DON EIA, and VERATOX kits (**Table 1**).

The antibodies in AGRAQUANT kits present moderate cross-reactivity for **DON** > DON-3-Glc > de-epoxy metabolite 1, whereas a low cross-reactivity was obtained for HT2-toxin > fusarenone X > 15-acetylDON. High relative cross-reactivity (> 100%) was observed against 3-acetylDON (cross-reactivity = 770 ± 83%). In contrast, no cross-reactivity was observed with diacetoxyscirpenol, nivalenol, T2-toxin, verrucarol, and zearalenone up to 2.0 μg/g of cereal.

As for DON EIA, the cross-reactivity of the antibodies was observed as follows: 3-acetylDON > nivalenol > DON-3-Glc > **DON** > de-epoxy metabolite 1 > fusarenone X. No cross-reactivity was observed with 15-acetylDON, diacetoxyscirpenol, T2-toxin, HT2-toxin, verrucarol, and zearalenone up to 1.9 μg/g of cereal.

In the case of VERATOX, the antibodies interact significantly against de-epoxy metabolite 1 > DON-3-Glc > **DON** > 3-acetylDON. No cross-reactivity was observed with 15-acetylDON, diacetoxyscirpenol, nivalenol, fusarenone X, T2-toxin, HT2-toxin, verrucarol, and zearalenone up to 1.0 μg/g of cereal.

Characterization of ROSA LF-DONQ Antibodies against Some Fusariotoxins. DON determination by the CHARM reader started at 0 μg/kg with an increment of 50 μg/kg intervals. The linearity procedure was difficult to achieve with several points within the same increment, but a large range of mycotoxin concentrations (from different increments) showed significant dose response profiles. Histograms (**Figure 2**) present the average of the responses assumed as DON detected by ROSA LF-DONQ (μg/kg of cereal) against the equivalent of mycotoxin levels (μg/kg of cereals).

For DON, 3-acetylDON, 15-acetylDON, de-epoxy metabolite 1, HT2-toxin, and DON-3-Glc, the dose response profiles are linearly regressed against the equivalent of DON concentrations (μg/kg of cereal) and yielded significant positive correlation coefficients. The cross-reactivity percents ($\text{slope}_{\text{mycotoxin}}/\text{slope}_{\text{DON}} \times 100$) were subsequently derived and summarized in **Table 2**. One could conclude that these mycotoxins showed significant cross-reaction with the antibodies. In contrast, no response of DON measurement was observed for diacetoxyscirpenol, fusarenone X, nivalenol, T2-toxin, verrucarol, and zearalenone, suggesting the absence of their cross-reaction with the antibodies.

Characterization of MYCONTROL DON Antibody against Some Fusariotoxins. *Verification of Dilution Linearity with DON Standards.* The dilution linearity test is summarized in **Table 3**. The deviation from overall mean values ranged between −10 and +10% and showed good linearity of the responses within the range of 140−860 μg of DON/kg of cereal. On the basis of this good linearity response, various concentrations of the tested mycotoxins were directly assayed via the AOKIN procedure. DON concentration above 860 μg/kg (tested high limit) can be measured quantitatively, via appropriate and precise dilution steps. It is noteworthy that good repeatability (CV < 5%) was also obtained.

Cross-Reactivity in MYCONTROLDON Antibody. Using DON standard, the dose response profiles are linearly regressed against the DON contents in solution (μg/kg), because all deviations lie between −10 and +10% from the mean normalized value. Linear regression analyses allow estimation of the cross-reactivity.

The dose response profiles assumed as DON measurements (μg/kg) via AOKIN spectrophotometer generated for DON, 3-aetylDON, DON-3-Glc, de-epoxy metabolite 1, T2-toxin, and HT2-toxin are depicted in **Figure 3**. Significant positive correlation coefficients have been derived and yielded the cross-reactivity percents ($\text{slope}_{\text{mycotoxin}}/\text{slope}_{\text{DON}} \times 100$) as summarized in **Table 2**. Note that high relative cross-reactivity (> 100%) was observed for 3-acetylDON (167 ± 10%). Marginal responses and nonsignificant correlation coefficients were obtained for diacetoxyscirpenol, nivalenol, fusarenone X, verrucarol, 15-acetylDON, and zearalenone, suggesting a minor presence or total absence of cross-reaction with the antibodies used in building this kit.

Comparison of Measured versus Declared Cross-Reactivity Values. Information about the cross-reactivity values was gathered from the manufacturers. **Table 4** presents the comparison of the declared values versus the measured values in the present study.

Most of the manufacturers did not report cross-reactivity values for de-epoxy metabolite 1, DON-3-Glc, diacetoxyscirpenol, HT2-toxin, verrucarol, and zearalenone in their validation dossier. As for 3-acetylDON, 15-acetylDON, fusarenone X, nivalenol, and T2-toxin, results are somewhat different from the values indicated by the manufacturers. With regard to AGRAQUANT, with declaration of cross-reactivity to 3-acetylDON of > 100%, our measurements showed that it was approximately 7.7 times

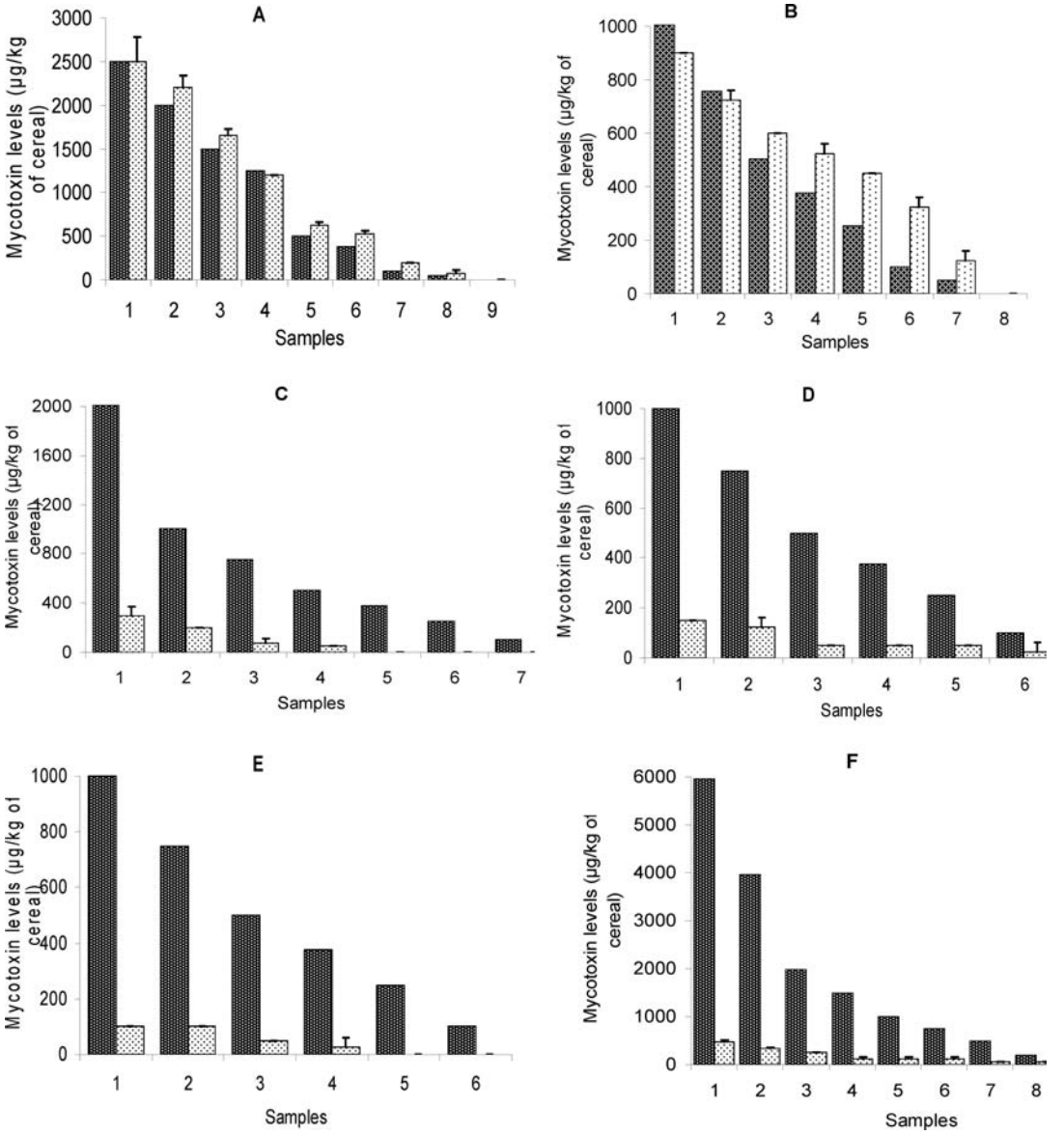

Figure 2. Responses assumed as deoxynivalenol contents (white-small confetti bar) via ROSA LF-DONQ (μg/kg) derived from the standards of deoxynivalenol (**A**), 3-acetylDON (**B**), 15-acetylDON (**C**), de-epoxy metabolite 1 (**D**), HT2-toxin (**E**), and deoxynivalenol-3-glucoside (**F**) expressed as equivalent concentrations (μg/kg of cereal, gray bar) according to the samples.

Table 2. Tested Mycotoxin Contents and Cross-Reactivity Responses via ROSA LF-DONQ and MYCONTROLDON Kits

	ROSA LF-DONQ			MYCONTROLDON		
	range of tested concentrations (μg/kg of cereal)	n[a]	cross-reactivity (%	range of tested concentrations (μg/kg of cereal)	n	cross-reactivity (%)
deoxynivalenol	0−2500	9	100 ± 3	157−785	6	100 ± 5
3-acetylDON[b]	100.8−1008	6	60 ± 2	40−806.4	7	167 ± 10
15-acetylDON[b]	100−2000	7	17 ± 4	402−1608	3	NR
de-epoxy metabolite 1[b]	100−1000	6	14 ± 2	204−1632	5	10 ± 3
HT2-toxin[b]	100−1000	6	13 ± 2	300−1200	6	8 ± 1
DON-3-Glc[b]	0−5952	10	8 ± 1	79−4762	8	22 ± 1
diacetoxyscirpenol	100−2000	7	NR	200−800	3	8 ± 1
fusarenon X	100−1000	6	NR	204−1632	4	4 ± 1
nivalenol	100−1000	6	NR	213−854	3	5 ± 3
T2-toxin	100−1000	6	NR	300−800	4	11 ± 3
verrucarol	500−2000	4	NR	182−1458	4	5 ± 1
zearalenone	0−1000	6	NR	210−840	3	NR

[a] n = number of samples analyzed in duplicate; NR = no cross-reaction. [b] ROSA LF-DONQ or MYCONTROLDON did not significantly differentiate this mycotoxin from DON.

higher. The observed differences are not unexpected because the results of cross-reactivity vary according to the variability and uncertainty of input data and the methods used for its assessment (*19*). For all of the examined commercial ELISA test kits [Ridascreen DON (provided by R-Biopharm), VERATOX DON 5/5, DON EIA, and AGRAQUANT], large differences existed between their experimental data and producers' information on cross-reactivity to acetylated DONs (*25*). The cross-reactivity to 15-acetylDON in line with producer's statement was negligible. Furthermore, setting the activity for DON as 100%, it was also found that the cross-reactivity may reach 930% for 3-acetylDON against 57.4% for nivalenol and 22.9% for 15-acetylDON (*23*). The potential contribution of acetylDONs to the overestimation of results generated by the DON ELISA test was previously discussed (*26*, *27*). The antibody used for examination showed cross-reactivity to 3-acetylDON and 15-acetylDON as high as 216 and 260%, respectively. The cross-reactivity to DON-3-Glc has been clearly proven (*25*). These authors have found a DON overestimation in beer analyzed via AGRAQUANT assay, up to 1000%, when taking into account the DON content as determined by LC-MS/MS, a reference method.

There is a need to officially update this information by international standardization bodies for validating analytical methods for mycotoxins. This validation should take into account some mycotoxins such as 3-acetylDON, nivalenol, DON-3-Glc, de-epoxy metabolite 1, fusarenone X, and HT2-toxin. The experiments in this study were performed in pure aqueous solution (water), and we suggest the cross-reactivity study be performed

Table 3. Dilution Linearity of the Responses via MYCONTROLDON Spectrophotometer to Various DON Concentrations

	normalized[a] tested DON concentration (μg/kg of cereal)				
dilution factor	trial 1	trial 2	average	CV (%)	deviation from overall mean value (%)
1	811.0	801.0	806.0	0.9	0.3
0.8	857.0	863.1	860.1	0.5	7.0
0.66	751.7	766.8	759.2	1.4	−5.6
0.375	832.0	854.1	843.1	1.9	4.9
0.25	720.4	764.4	742.4	4.2	−7.6
0.2	841.0	784.0	812.5	5.0	1.1
overall mean (μg/kg)			803.9		
SD (μg/kg)			45.92		

[a] Normalized tested DON concentration (μg/kg of cereal) = DON contents via AOKIN/dilution factor.

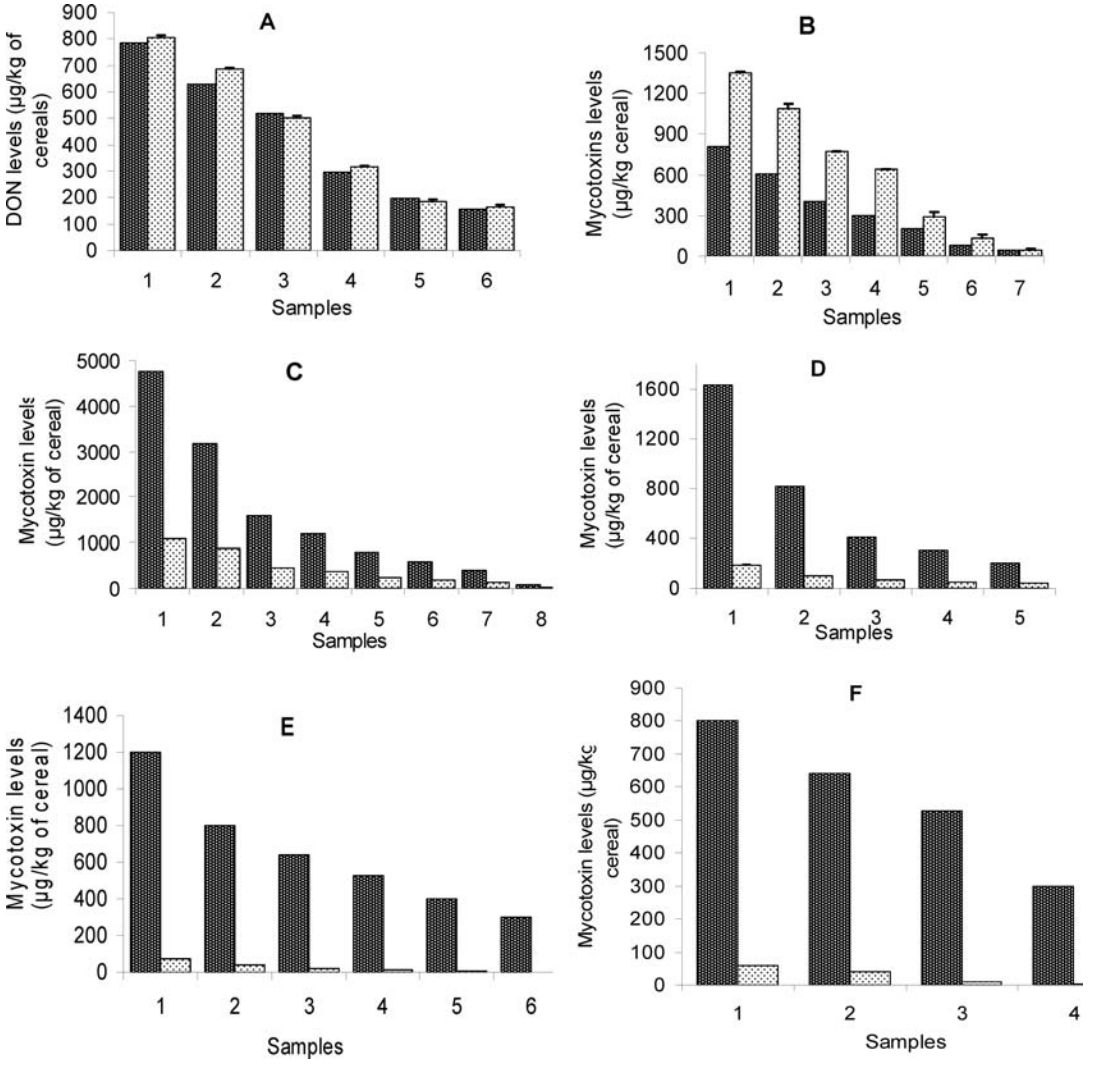

Figure 3. Responses assumed as deoxynivalenol contents (white-small confetti bar) via MYCONTROLDON (μg/kg of cereal) derived from the standards of deoxynivalenol (**A**), 3-acetylDON (**B**), deoxynivalenol-3-glucoside (**C**), de-epoxy metabolite 1 (**D**), HT2-toxin (**E**), and T2-toxin (**F**) expressed as equivalent concentrations (μg/kg of cereal, gray bar) according to the samples.

Table 4. Comparison of Measured versus Declared Cross-Reactivity Values according to the Tested Kits

kit	3-acetylDON	de-epoxy metabolite 1	DON-3-Glc	fusarenoneX	15-acetylDON	nivalenol	diacetoxy scirpenol	T2-toxin	HT2-toxin	verrucarol	zearalenone
DON EIA											
measured[a] (%)	230 ± 1	84 ± 2	115 ± 6	32 ± 2	0	198 ± 11	0	0	0	0	0
declared[b] (%)	96	NA	NA	NA	<0.1	40	NA	NA	NA	NA	NA
VERATOX											
measured (%)	40 ± 4	212 ± 16	157 ± 2	0	0	0	0	0	0	0	0
declared (%)	105	0	0	0.4	7	3.8	NA	NA	NA	NA	NA
AGRAQUANT											
measured (%)	770 ± 83	35 ± 1	52 ± 2	0.7 ± 0.5	2 ± 1	0	0	0	2 ± 0.1	0	0
declared (%)	>100	NA	NA	0%	NA	0	NA	0	NA	NA	NA
MYCONTROL DON											
measured (%)	167 ± 10	10 ± 3	22 ± 1	4 ± 1	1 ± 1	5 ± 3	8 ± 1	11 ± 3	8 ± 1	5 ± 1	0
declared (%)	300	NA	NA	<0.1	5	<0.1	<0.1	<0.1	<0.1	NA	NA
ROSA LF-DONQ											
measured (%)	60 ± 2	14 ± 2	8 ± 1	0	17 ± 4	0	0	0	13 ± 2	0	0
declared (%)	200	NA	NA	NA	30	0	NA	NA	NA	NA	0

[a] Measured = results obtained in the present study. [b] Declared = results supplied by manufacturer. NA, data not available.

with spiked matrix extract to evaluate the background noise of each specific matrix. This may allow checking meanwhile the matrix effect on cross-reactivity results as previously performed (*25*). It is very well-known that mycotoxin identification and quantification can be hampered by matrix effects due to unknown matrix components that may contribute to false-positive results (*25*, *28*).

Major Tested Fusariotoxins Interacting with the Commercial Tested DON Antibodies. High cross-reactivity is considered to be a drawback because it does not allow checking the compliance with legal norms (*14*). Nevertheless, it may allow an idea about the whole amount of the target compound together with other detectable analogues.

As the same panel of mycotoxins is checked for all tested kits, the results showed the major cross-reacting toxins with most commercial DON antibodies. Humans and animals are likely to be simultaneously exposed to several contaminants, present in the environment or in the foods. This seems to be particularly the case for several mycotoxins that can be present together and may have additive or synergistic effects. Consideration of the likelihood of co-occurrence of the tested toxins, the closeness of their chemical structure to DON in combination with their toxicity should be taken into account in interpreting the cross-reactivity results. Due to larger differences in structure, diacetoxyscirpenol, T2-toxin, HT2-toxin, verrucarol, and zearalenone present low or marginal cross-reactivity toward the DON antibody used in manufacturing the commercial kits. 15-AcetylDON and fusarenone X showed moderate cross-reactivity against the DON antibody and presented low incidence in cereals and cereal products. Interestingly enough, cross-reactivity with de-epoxy metabolite 1 was observed, but this should not be a problem because the de-epoxy metabolite 1 level has been triggered by the biological transformation of DON, allowing a decrease of the toxicity of DON. In cell toxicity tests, de-epoxy metabolite 1 resulting from the de-epoxidation was found to be 24 times less toxic than the parent molecule DON because the 12,13-epoxide ring, essential for the toxicity of the trichothecenes, has been removed (*29*). One could state, therefore, the relevant importance or urgency for further investigation into this metabolite. In contrast, 3-acetylDON, DON-3-Glc, and nivalenol showed strong cross-reactivity toward the antibody. Some of these native or masked toxins could coexist in cereal products and other foods or feedstuffs. Therefore, analytical results produced by these ELISA kits can be wrongly interpreted. DON-3-Glc can be transformed to DON and the potential contribution of this major DON conjugate in overestimating (target) DON levels has to be taken into consideration. ELISA is employed for screening purposes, and positive results obtained by this technique have to be confirmed by a more specific method, such as LC or GC methods.

Thanks to the cross-reactivity results, relevant information (determination of "total" DON content in cereal and cereal-based products) may be rapidly obtained through screening. A consumer's risk of exposure could be realistically assessed by taking into account the occurrence of "unknown" cross-reactive compounds (i.e., acetylDONs, DON-3-Glc, and nivalenol) together with DON.

Quantification of DON by immunoassays is complicated by cross-reaction with 3-acetylDON and 15-acetylDON (*30*). These compounds may lead to an overestimation, especially in the uncertainty area around the legal limits. The maximum residue levels (MRLs) recommended by European Commission (EC) regulation no. 1881/2006 (*31*) is 1250 μg/kg for unprocessed cereals other than durum wheat, oats, and maize, 750 μg/kg for cereal flour, bran, and germ, and 200 μg/kg for processed cereal-based baby and infant foods. In animal feedings, the MRL of 8000 μg of DON/kg was recommended by EC regulation no. 2006/576/EC (*32*) for cereals and cereal products (with the exception of maize byproduct) and 12000 μg of DON/kg of feed for maize byproduct. For evaluation of the compliance with the EU or international regulatory limits, we recommend analysis with another sophisticated method, especially for the uncertainty area around the legal limits and for samples showing results above the norms. From a cost perspective, it is desirable for a rapid screening method to give as few false-positive results as possible, because confirmation tests are necessary after each positive result. A high number of false-positive results becomes, therefore, too expensive.

To determine DON together with its related derivatives, preliminary acetylation of toxins in the extracts followed by determination of the sum of DON acetyl derivatives was proposed (*33*). The specificity of antibodies to nivalenol, the toxicity of which can be compared to that of DON, requires an additional derivatization of the toxin (*34*). The simultaneous determination of nivalenol and DON was recently proposed through competitive direct and indirect ELISA formats by developing a monoclonal antibody with the nivalenol−glycine immunogen (*23*).

It is obvious that the tested rapid methods highlighted the existence of antibody cross-reactions. Data obtained in this work are somewhat different from the values declared by the manufacturers. Moreover, this study stresses the need to test the antibodies for specificity against some important native mycotoxins

such as nivalenol, conjugated products of DON such as DON-3-Glc, and important derivatives such as 3-acetylDON. These mycotoxins should be strictly checked by commercial kit producers via regulatory organizations. For the validation of rapid tests, we suggest standardizing the list of requested mycotoxins at the international level. As for DON kits, 3-acetylDON, nivalenol, DON-3-Glc, de-epoxy metabolite 1, and fusarenone X should be included. There is a need to control each batch of the produced kit by the manufacturer or certifying body as far as the cross-reactivity is concerned. Despite these cross-reactions, the immunochemical methods mentioned are still valuable for quantitative screening and even for initial exposure assessment in situations when there are practical and economical reasons not to use sophisticated methods.

ABBREVIATIONS USED

ELISA, enzyme-linked immunosorbent assay; LFD, lateral flow devices; FPIA, fluorescent polarization immunoassays; CV, coefficient of variation; B/B_0, relative absorbance.

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