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Feasibility of Immunodiagnostic Devices for the Detection of Ricin, Amanitin, and T-2 Toxin in Food

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ABSTRACT

Qualitative and quantitative comparisons were conducted of commercially available immunodiagnostic devices for the detection of three select agents with oral LD_{50} values $\geq$0.1 mg/kg of body weight. Ricin (oral $LD_{50} > 1$ mg/kg), amanitin (oral LD_{50} approximately 0.1 mg/kg), and T-2 toxin (oral $LD_{50} > 1$ mg/kg) were spiked into beverages, produce, dairy, and baked goods and assayed using commercially available enzyme-linked immunosorbent assays (ELISAs) and lateral flow devices. In all cases, the commercial diagnostic kits successfully detected all three select agents at concentrations below what might be a health concern. The considerable difference between the limit of detection of the immunodiagnostic devices employed (typically $\leq$0.020 μg/g) and the amount of the select agent necessary to pose a health threat in a single serving of food facilitated the design of protocols for the high throughput screening of food samples. These protocols entailed simple extraction methods followed by sample dilution. Lateral flow devices and sandwich ELISAs for the detection of ricin had no significant background problems due to the food matrices. Competitive ELISAs, which typically have unacceptably high background reactions with food samples, successfully detected amanitin and T-2 toxin.

The ability to detect toxins quickly and accurately and with sufficient sensitivity is critical to ensure a safe food supply. The heterogeneous nature of food matrices complicates diagnostic assays. Immunodiagnostic assays circumvent many matrix problems by relying on the specificity of antibodies to recognize unique epitopes. Food matrices pose a problem because of the ability of some of the components in the food to bind to the toxin and mask the epitope. This decreases the ability of the diagnostic assay to recognize the toxin but may not reduce the biological activity of the toxin. Furthermore, the food matrix may interact with the diagnostic components and reduce the effectiveness of the assay. An example of such an interaction is the nonspecific binding of a food component to the wells of the microtiter plate used in an enzyme-linked immunosorbent assay (ELISA). In a sandwich ELISA, the nonspecific binding to the wells decreases the sensitivity and increases the limit of detection. In contrast, nonspecific binding to the wells in a competitive ELISA may result in false-positive results or inflated estimates of antigen.

Several methods can be employed to circumvent problems of nonspecific binding by food components. One of the simplest involves exploiting the difference in the binding affinities of the epitope versus the nonspecific binding components. Provided the two affinities are sufficiently different (i.e., two orders of magnitude), dilution of the sample should have a much greater impact on the nonspecific binding (background) than on the detection of the antigen.

One gauge of the amenability of the assay to being adapted for the handling of complex samples such as food matrices is the magnitude of the difference between the limit of detection of the diagnostic assay for the toxin in a simple homogeneous noninterfering medium (i.e., buffer) versus the oral LD_{50}. The smaller the difference, the more likely that the food matrix will inhibit the effectiveness of the assay. Similarly, the greater the difference, the more flexibility is possible in the operation of the assay and the preparation of the food sample for analysis. In this study, the effectiveness of commercially available immunodiagnostic assays for the detection of three select agents with oral LD_{50} values equal to or greater than 0.1 mg/kg of body weight were evaluated.

Ricin is a phytotoxin derived from castor beans, *Ricinus communis.* Despite a low toxicity level (oral LD_{50} estimated at >1 mg/kg of body weight *(8)*), the notoriety of ricin in the media has led its classification as a potential threat agent *(3).* Ricin is a heterodimeric protein consisting of two subunits *(13, 15).* The A chain is a ribosome inactivating protein, which catalyzes the depurination of adenine A4324 of 28S rRNA *(14, 21, 22).* The B chain is a lectin and facilitates the uptake of the toxin into the cell. *R. communis* also contains a second toxin, a hemagglutinin (RCA-120) that has a molecular weight of 120,000, and there is 93 and 84% homology between the A and B chains of the two toxins, respectively *(2, 16, 17).*

α-Amanitin is one of a group of thermostable bicyclic octapeptides, the amatoxins. It is the major toxin of the extremely poisonous mushrooms *Amanita phalloides* (death cap), *A. verna* (destroying angel), *A. virosa, A. ocreata, A.*

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tenuifolia, and other *Amanita* species. The first three species account for more than 90% of clinically relevant mushroom intoxication cases in Europe and North America *(19, 20).* The primary action of these toxins is inhibition of nucleoplasmic RNA polymerase II. The result is the arrest of protein synthesis and cellular necrosis leading to severe acute hepatitis *(5).* Amanitin also acts as a direct nephrotoxin. The oral LD_{50} of α-amanitin in humans is estimated to be 0.1 mg/kg of body weight (or the equivalent of one mushroom for a 75-kg adult) *(1).* α-Amanitin is not as readily available as other purified natural toxins; however, its extreme toxicity, water solubility, and heat stability make this toxin an agent of concern.

T-2 toxin (fusariotoxin, 4β,15-diacetoxy-3α,dihydroxy-8 α-[3-methylbutyryl-oxy]-12,13-epoxytrichothec-9-ene) is a trichothecene mycotoxin produced by species of the genus *Fusarium* and has been extensively reviewed by Canady et al. *(4).* T-2 toxin is heat stable and easily absorbed through the skin. Oral LD_{50} values for mice and chickens have been reported as 2 to 10 mg/kg of body weight *(6, 10, 12, 18).* Complicating the assignment of a specific LD_{50} value for T-2 toxin is its ability to induce debilitating effects even at sublethal doses.

The following immunology-based diagnostic kits for the detection of ricin, amanitin, and T-2 toxin in environmental samples were evaluated: lateral flow devices (LFDs) from a government supplier for the detection of ricin, LFDs and ELISAs manufactured by Tetracore, Inc. (Gaithersburg, Md.) for the detection of ricin, the ALPCO ELISA kit for the detection of amanitin, and the Ridascreen kit (R-Biopharm, Damstadt, Germany) for the detection of T-2 toxin. The limits of detection for these kits were listed by the manufacturers as $\leq$0.050 μg/ml, making them much more sensitive than necessary to detect a lethal dose of ricin, amanitin, or T-2 toxin in a serving of food. Because of this high degree of sensitivity, it was possible to develop protocols that minimized extraction procedures and worked with all food matrices examined.

The specificities of the various ricin assays for the subunits of ricin and the cross-reactions between ricin and the agglutinin RCA-120 and another plant derived RIP-II, abrin C, were compared. The government supplier and the Tetracore immunodiagnostic devices were specific for different subunits and thus complemented each other.

MATERIALS AND METHODS

Reagents. Ricin, A chain, B chain, and the agglutinin RCA-120 were obtained from Vector Laboratories (Burlingame, Calif.) and were used without further purification. Substocks of ricin were prepared at 250 μg/ml and stored or used for a maximum of 3 weeks. Additional dilutions were made immediately before use. Abrin C and α-amanitin were obtained from Sigma (St. Louis, Mo.). The abrin C was supplied as a solution and stored at 4°C without further purification. Stock solutions of α-amanitin were prepared at 1,000 μg/ml in water and stored frozen in light-protected vials. Foods were obtained fresh from a local market and stored as recommended by the manufacturer.

Assays. Ricin LFDs were obtained from Tetracore and a government supplier (P. Emanuel, JPEO-CBD Office, and R. Bull, BDRD, U.S. Navy). Sample extraction was conducted for liquid matrices by making a fivefold dilution with 200 mM sodium phosphate (pH 6.8) and for solid matrices by washing with 25 ml of 200 mM sodium phosphate (pH 6.8) followed by 1:1 dilution with phosphate-buffered saline (PBS; Sigma catalog no. P3813). A 150-μl volume of the extracted sample was loaded onto the LFD, and results were obtained after 30 min at room temperature (22°C) using the Guardian Strip Reader (Alexeter Technologies, Wheeling, Ill.) and reported as the measured reflectance absorbance value. Because the government-supplied LFDs rely on visual scoring, the results were manually categorized as either positive or negative, without quantification.

The ELISA for the detection of ricin was obtained from Tetracore and used as recommended by the manufacturer. The only modifications were the incorporation of an eight-point calibration curve as part of each ELISA plate and the reading of plates at both 405 and 650 nm after 26 min of incubation at 37°C. All samples were extracted as described for the LFD analyses.

ELISA kits for the detection of amanitin were obtained from ALPCO (Windham, N.H.). The analyses of food samples spiked with 1 μg amanitin per g of food (or ml of beverage) were conducted according to the manufacturer's recommendations. Preliminary results revealed that the extraction of 5 g of food (spiked with amanitin at either 1 μg/g or 5 μg/g) with 100 ml of water resulted in an amanitin concentration within the range of the standard curve and suitable for quantification.

ELISA kits for the detection of T-2 toxin were obtained from R-Biopharm. The analyses of food samples spiked with 0.2 or 1 μg of T-2 toxin per gram of food were conducted according to the manufacturer's recommendations. To avoid overloading the assay and to allow quantification, the samples were diluted 100-fold during analysis with 35% methanol in water.

Sample spiking. All samples except solid milk chocolate were prepared by spiking with the select agent in PBS for ricin, in water for amanitin, and in methanol for T-2 and allowing the agent to fully absorb into the food prior to analysis. Solid milk chocolate containing ricin was prepared by melting 3 g of milk chocolate in aluminum containers and mixing in 1.25, 3.75, 12.5, 50, and 100 μg of ricin. The chocolate was then stored at 4°C until use. For analysis, 0.3 g of the chocolate was melted for 1 min at 65°C, 3 ml of 200 mM sodium phosphate, pH 6.8, was added and mixed for 30 s, and 200 μl of the mixture was diluted with 1.8 ml of 200 mM sodium phosphate, pH 6.8, and then diluted 1:1 with PBS prior to analysis.

Data analysis. The fractional responses (FRs) (where response was absorbance at 405 or 650 nm) observed for the ricin samples were calculated as the [(response of the food sample spiked with ricin) − (response of the food sample spiked with buffer)] ÷ [(response of ricin in buffer at the same concentration as the food sample) − (response of buffer)]. The responses observed using the amanitin and T-2 toxin ELISAs were expressed in the units of the standards supplied with the kits (ppm of toxin).

RESULTS

Ricin: specificity. The reactivity of the two LFDs and the ELISA for the detection of the ricin A and B chains and the cross-reactivity with the agglutinin RCA-120 and the RIP-II abrin C were examined (Table 1). The LFDs from the government supplier displayed a preference toward the ricin A chain and significant cross-reactivity with the agglutinin RDA-120 and the B chain subunit. In contrast, the LFD manufactured by Tetracore interacted pref-

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TABLE 1. *Cross-reactivity of government-supplied LFDs for the detection of ricin*

Ricin concentration in sample (μg/ml)[a]	Cross-reactivity with: Ricin A chain	Ricin B chain	RCA-120
0	−	−	−
0.005	−	−	−
0.0125	−	−	−
0.025	−	−	−
0.05	+	−	−
0.125	+	−	−
0.25	+	−	+
0.5	+	+	+
1	+	+	+
2.5	+	+	+
5	+	+	+
25	+	+	+
50	+	+	+
125	+	+	+
250	+	+	+
500	+	+	+

[a] Concentrations after dilution with PBS. All samples were prepared in 200 mM sodium phosphate (pH 6.8) and diluted 1:1 with PBS immediately prior to analysis.

erentially with the B chain of ricin with significant cross-reactivity with the agglutinin RCA-120 and the A chain (Fig. 1A). Neither LFD displayed cross-reactivity with abrin C. Titration of the response of the Tetracore LFD with ricin indicated a maximum response at 0.250 μg/ml, whereas the maximum response with agglutinin RCA-120 was obtained at 50 μg/ml (Fig. 1B).

The ELISA displayed a preference for the ricin B chain (Fig. 2). In contrast to the LFDs, cross-reactivity with the agglutinin RCA-120 was less than that observed with the A chain. Neither the LFDs nor the ELISA displayed cross-reactivity with abrin C (Figs. 1 and 2).

Ricin: hook effect. The LFDs manufactured by Tetracore and by the government supplier both displayed a decreased response with high concentrations of ricin; commonly referred to as a hook effect *(7, 11).* Two different lots of LFDs manufactured by Tetracore displayed similar hook effects. The presence of ricin was correctly detected (responses ≥ 0.01 units) at concentrations ranging from 0.005 to 500 μg/ml in PBS, with maximum sensitivity at 0.250 μg/ml (see Fig. 3). In contrast, LFDs from the government supplier gave variable results. The government-supplied LFDs packaged in bulk were not able to detect ricin at 5,000 μg/ml (false negative), and prolonged developing times were necessary for the detection of 500 μg/ml. No false-negative results were obtained with a second lot of LFDs that were individually wrapped (data not shown). The lack of a strip reader for the LFDs from the government supplier made it impossible to quantify the intensity of the responses.

Ricin: liquid food matrices. Figure 4 illustrates the comparison of the results obtained using LFDs manufac-

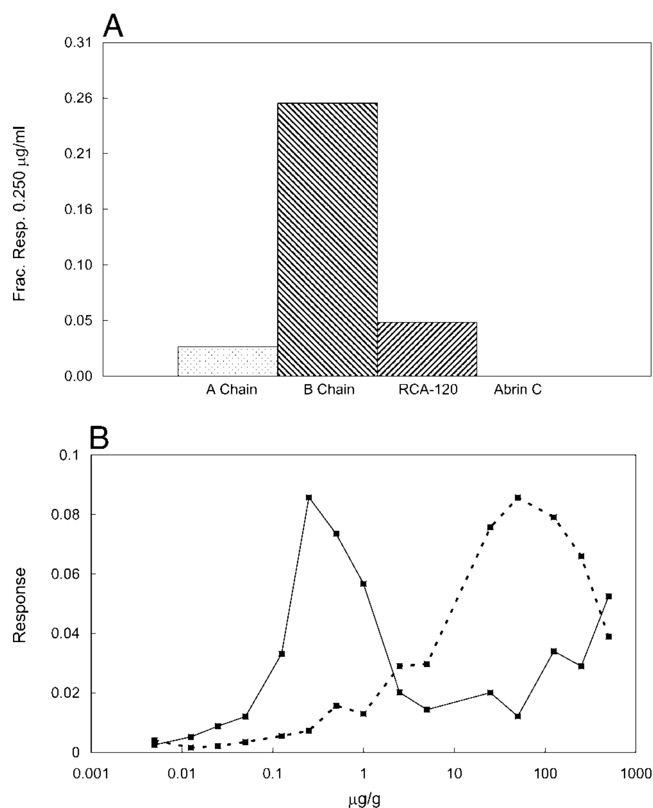

FIGURE 1. *Cross-reactivity of Tetracore LFDs with ricin subunits, agglutinin RCA-120, and abrin C. All samples were prepared by diluting the ricin into 200 mM sodium phosphate (pH 6.8) to make a 2× solution. Immediately prior to analysis, the sample was diluted 1:1 with PBS. (A) Fractional response at 0.250 μg/g (ppm) of A chain (▫), B chain (▧), agglutinin RCA-120 (▥), and abrin C (at background) (▨). (B) Response of various concentrations of ricin (——) and RCA-120 (– – –).*

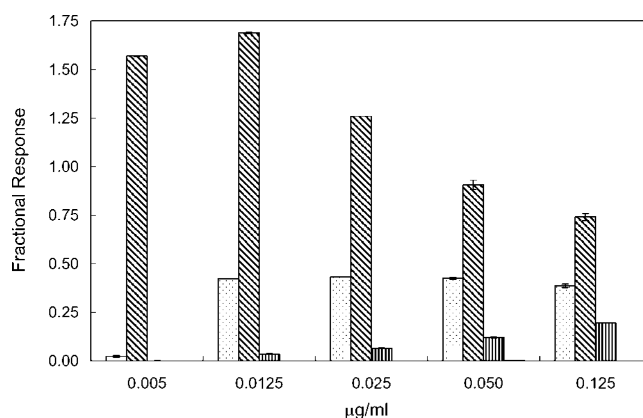

FIGURE 2. *ELISA cross-reactivity with ricin A chain (▫), ricin B chain (▧), agglutinin RCA-120 (▥), and abrin C (at background) (▨) expressed as the fractional response relative to the response expected for a comparable concentration of ricin in PBS. The concentrations listed are those following complete sample extraction. The error bars indicate the range of the results obtained between the duplicate analyses.*

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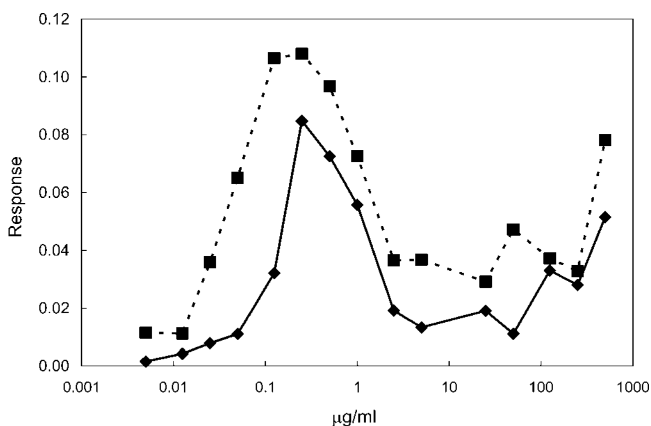

FIGURE 3. *Hook effect in the Tetracore LFD response with ricin. All samples were prepared at a 2× concentration in 200 mM sodium phosphate (pH 6.8) and diluted with PBS prior to analysis. Lot A (———); lot B (– – –).*

tured by Tetracore and those obtained with the ELISA for the detection of ricin at 0, 0.1, 0.25, 0.5, and 1 μg/ml (final concentrations following all dilutions and extraction steps were 0, 0.01, 0.025, 0.050, and 0.100 μg/ml) in four fruit juices, a vegetable juice, two dairy beverages, and soda. The data are presented as the response observed versus the response observed for the same amount of ricin in buffer. An FR value of 1 indicates 100% recovery and means that the matrix had no effect on the assay. FR values of <1 indicate the loss of ricin, and FR values of >1 indicate a ricin concentration greater than expected. An FR value of >1 can be due to calculation error or to a reduction in the volume of solvent by the sample. An example of the former would be the subtraction of a background response that is a major part of the total signal, as may occur at low concentrations of ricin. Most of the responses with the LFDs that yielded FR values >1 were at the two lowest concentrations of ricin examined (0.010 and 0.025 μg/ml after extraction), with the background contributing a large proportion of the signal. An example of the latter mechanism for generating a FR value >1 would be the hydration of a matrix component.

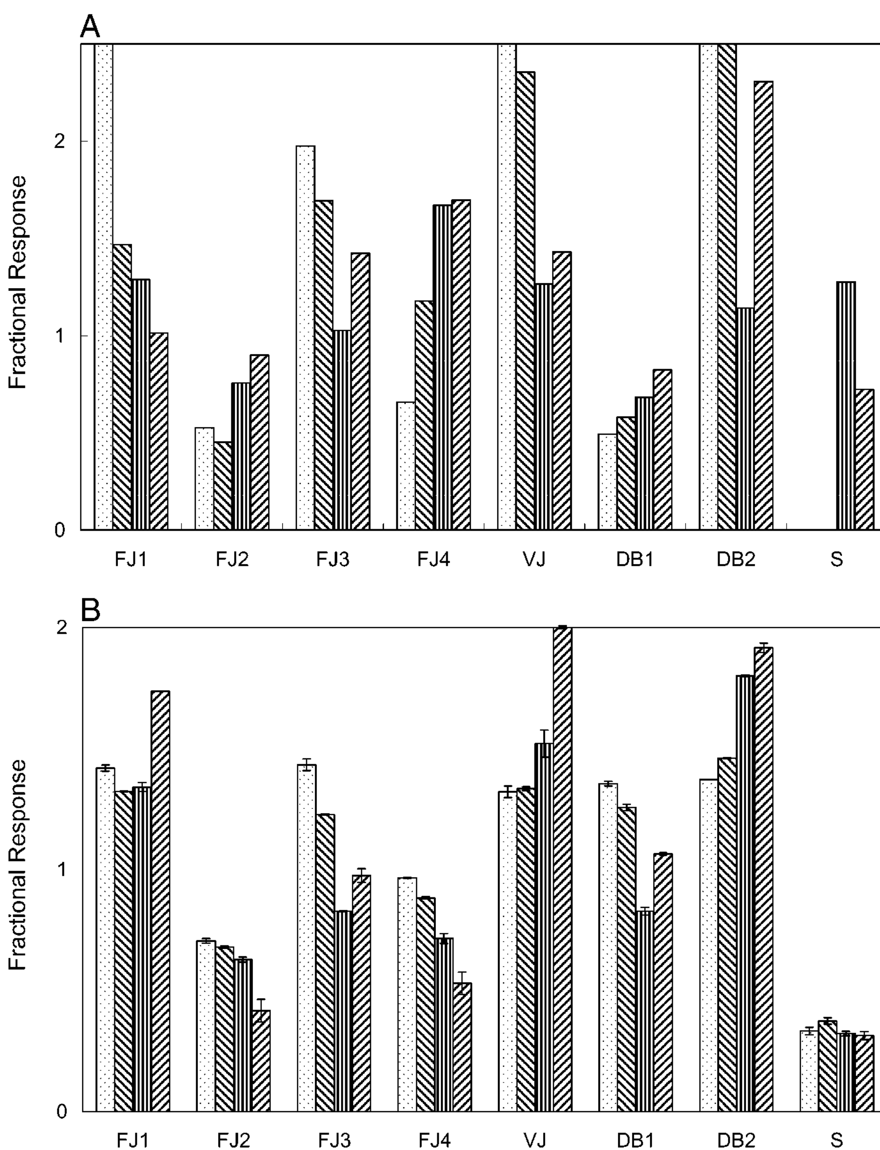

FIGURE 4. *LFD (A) and ELISA (B) analyses of 0.5 ml of fruit juices (FJ1, FJ2, FJ3, and FJ4), vegetable juice (VJ), dairy beverages (DB1 and DB2), and soda (S) spiked with ricin at 0 (FR = 1 by definition), 0.1 (▒), 0.25 (▧), 0.5 (▥), and 1.0 (▨) μg/ml. After 30 min of incubation at room temperature (22°C), the pH of the sample was neutralized by the addition of 2 ml of 200 mM sodium phosphate (pH 6.8). The sample was then diluted 1:1 with PBS and analyzed. The final concentrations of ricin at the time of analysis were 0, 0.010, 0.025, 0.050, and 0.100 μg/ml. The error bars indicate the range of the results obtained between duplicate analyses of the samples.*

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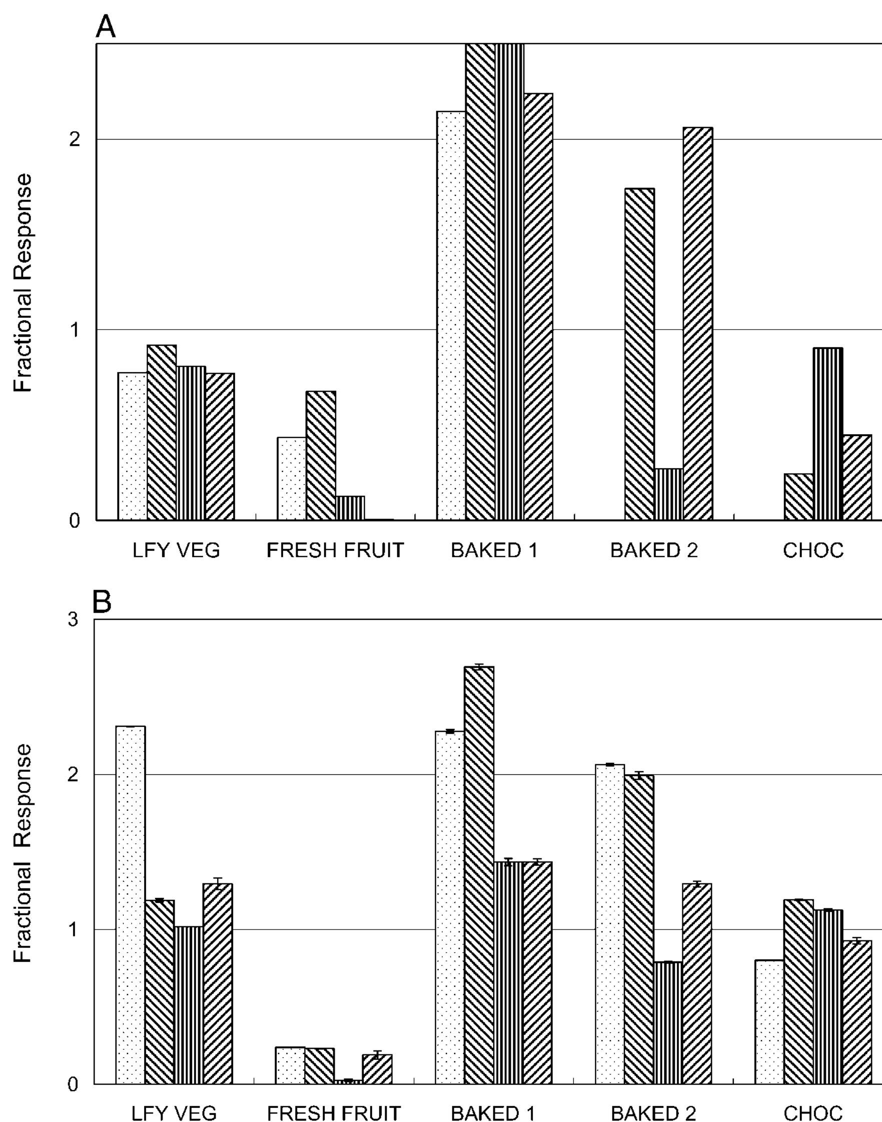

FIGURE 5. *LFD (A) and ELISA (B) analyses of 5 g of leafy vegetable (LFY VEG), fresh fruit (FRESH FRUIT), baked goods (BAKED 1, BAKED 2), and chocolate (CHOC) spiked with 0 (FR = 1 by definition), 0.5 (⚃), 1.25 (▧), 2.5 (▥), or 5 (▨) μg of ricin and incubated at room temperature (22°C) for 3 h. The leafy vegetable, fresh fruit, and baked goods were washed with 25 ml of 200 mM sodium phosphate, and washings were diluted 1:1 with PBS. The chocolate samples were prepared by mixing 1.25 (⚃), 3.75 (▧), 12.5 (▥), or 50 (▨) μg of ricin into 3 g of melted chocolate; mixtures were stored at 4°C until use. Extracts were obtained by heating 0.3 g of the chocolate-ricin mixture at 65°C for 1 min, immediate addition of 3 ml of 200 mM sodium phosphate, 30 s of mixing, dilution of 200 μl of the mixture with 1.8 ml of 200 mM sodium phosphate (pH 6.8), and final 1:1 dilution with PBS.*

The ELISA correctly detected the presence of ricin in all samples with FR values closer to 1 than those obtained with the LFDs. The seven samples that yielded unusually high FR values with the LFDs yielded acceptable FR values with the ELISA. This finding is consistent with the greater sensitivity of the ELISA to low concentrations of ricin and the smaller contribution of the background response to the signal, with less variance.

Ricin: solid food matrices. The FR values obtained using LFDs manufactured by Tetracore and using the ELISA for various solid food matrices are compared in Figure 5. As observed with the liquid samples, the ELISA successfully detected ricin in all spiked samples, but the LFDs were unable to detect ricin in several of the samples with low concentrations of ricin. The FR values for baked goods were repeatedly >1. All four samples of baked goods 1, analyzed by ELISA or LFD, had FR values >1. Three of four of the samples of baked goods 2 had FR values >1 in the ELISA, and two of three had FR values >1 with the LFDs. These reproducible observations of FR values >1 may reflect hydration of the baked good or a decrease in solvent volume with a resultant increase in ricin concentration. Shampoo samples spiked with ricin also displayed FR values >1, probably because of hydration of the detergent (data not shown).

Low FR values were obtained for fresh fruit, and the variable response was not correlated with the amount of ricin spiked onto the sample. In preparing these samples, some were spotted with ricin on the top of the fruit at the point of attachment to the stem. Because the B chain is a lectin, the ricin may have bound tightly to the sugar in the fruit, making the extraction (washing) less efficient. To test this hypothesis, fruit samples were spotted with 1.25, 2.5, and 5 ng of ricin on the side or the top at the point of attachment to the stem. The mean (±SD) FR value of the fruit spotted on the side was 0.42 ± 0.04, whereas fruit spotted on the top had a progressive increase in FR values with concentration (0.07, 0.25, and 0.42). A progressive increase is characteristic of a finite number of binding sites sequestering (absorbing) the ricin, as would be expected to occur at the junction with the stem due to binding to the sugar in the flesh of the fruit.

The FR values for the leafy vegetable and solid milk chocolate were more variable with the ELISA than with the

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TABLE 2. *Detection of amanitin in various food products using the ALPCO ELISA test kit*

Commodity sample	Amanitin concentration (ppm) detected after spiking with[a]:		
	BKGD	1 μg/g	5 μg/g
Breaded seafood	0.0	0.9	2.6
Canned fish	0.2	0.7	3.0
Water from canned fish	0.2	0.8	2.7
Canned vegetables 1	0.1	0.3	5.7
Canned vegetables 2	0.2	1.2	4.2
Cereal	0.2	1.2	3.2
Cookies	0.0	0.8	3.0
Corn meal	0.1	1.4	2.7
Crackers	0.0	2.2	4.6
Dairy beverage 1	0.0	0.9	3.8
Dairy beverage 2	0.3	0.9	5.4
Dairy beverage 3	0.0	1.1	4.2
Dairy product 1	0.0	1.4	4.5
Dairy product 2	0.0	1.1	3.6
Frozen dairy product	0.0	0.6	2.6
Fruit juice 1	0.0	0.8	5.0
Fruit juice 2	0.4	1.1	5.6
Fruit juice 3	1.4	2.2	6.1
Meat	0.2	1.4	5.9
Mushrooms	0.1	1.3	3.7
Pasta	0.0	1.9	3.5
Salty snack	0.3	2.2	4.6
Soda 1	0.4	1.6	2.6
Soda 2	0.3	1.0	5.4
Vegetable 1	0.7	0.7	5.4
Vegetable 2	0.1	1.5	4.5

[a] 5-g samples were spiked with 1 and 5 μg/g (ppm) amanitin. BKGD, background.

LFDs. The basis for this variability is not understood and may reflect artifacts associated with these particular matrices. Unless sufficiently diluted, solid milk chocolate samples can cause problems in ELISAs because of high levels of nonspecific binding to the plate wells. However, the solid milk chocolate samples that did not contain ricin yielded normal background responses.

Amanitin. The results of the ALPCO ELISA for amanitin in various food matrices are presented in Table 2. Amanitin was detected in all commodities that were spiked with amanitin at 1 and 5 μg/g. The background responses, i.e., those of samples that did not contain amanitin, varied by commodity, with most less than the equivalent of 0.5 ppm.

T-2 toxin. The results of the Ridascreen analysis for T-2 toxin in various food matrices spiked at 0.2 and 1 μg/g are presented in Table 3 along with the background responses. The background responses of food samples not spiked with T-2 toxin were significant and varied up to the equivalent of 0.1 ppm, with most between 0.03 and 0.06 ppm. As the data indicate, the kit successfully detected T-2 toxin in all spiked samples.

DISCUSSION

All immunodiagnostic devices were successfully adapted to detect their respective select agents in food samples at concentrations less than what would pose a health concern. For ricin detection, there were marked differences between the LFDs and the ELISA. The LFDs provided a fast user-friendly diagnostic assay that did not require any specialized equipment. However, the LFDs also had a higher failure rate for detection of very low concentrations of ricin (much less than the human lethal dose). The LFDs also displayed a decreased response at high ricin concentrations

TABLE 3. *Detection of T-2 toxin in food using the R-Biopharm Ridascreen test kits*

Commodity sample	T-2 toxin concentration (ppm) detected after spiking with[a]:			
	0.2 μg/g		1 μg/g	
	BKGD	Response	BKGD	Response
Canned soup	0.01	0.23	ND	ND
Canned mushrooms	0.01	0.22	0.07	0.74
Juice from canned mushrooms	0.04	0.30	0.05	0.70
Canned vegetable	0.05	0.45	0.05	0.70
Juice from canned vegetable	0.09	0.23	0.06	0.63
Dairy beverage 1	0.00	0.26	ND	ND
Dairy beverage 2	0.04	0.27	ND	ND
Dairy beverage 3	0.01	0.23	ND	ND
Dairy beverage 4	0.05	0.18	0.09	0.81
Frozen dairy product	0.10	0.45	0.04	0.21
Fruit juice 1	0.00	0.08	ND	ND
Fruit juice 2	0.00	0.17	ND	ND
Fruit juice 3	0.00	0.19	ND	ND
Soda 1	0.03	0.29	ND	ND
Soda 2	0.00	0.26	ND	ND
Vegetable juice	0.03	0.20	0.03	0.14

[a] Samples were spiked with 0.2 or 1 μg/g T-2 toxin. BKGD, background; ND, not determined.

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(hook effect), which may complicate detection and quantitation; however, the inclusion of an additional dilution step in the protocol resolved the problem. Quantitation was a problem with LFDs because of the need for strip readers, which were available only for LFDs manufactured by Tetracore. The background signal represented a large proportion of the LFD response to ricin and thus increased the variability in the data. Thus, use of LFDs should be limited to qualitative analyses. In contrast, the ELISA successfully detected the presence of ricin in all samples and was readily amendable to quantitative analysis. However, the ELISA required the use of specialized equipment and was best conducted in a laboratory setting by a trained operator. Thus, the LFDs are suitable for use as field deployable qualitative initial screening tests with ELISAs employed for confirmatory purposes with samples that display either a positive or a questionable response.

Limits of detection and sampling protocol. In designing protocols for the detection of toxins in food matrices, the oral LD_{50} value in humans (estimated at 1 to 20 mg/kg of body weight for ricin *(8)*) played an important role in adapting the assay and placed constraints on acceptable changes on the limits of detection. A conservative permissible lower limit of detection for ricin was estimated at 10 μg/g for adults, based on 10 mg in a 1-kg serving of food, and 4 μg/g for children, based on 1 mg in an 8-oz serving. Both LFDs and the ELISA were consistently effective at detecting ricin at these concentrations. The only time LFDs failed to yield a positive response at ricin concentrations deemed a health risk was when false-negative results were obtained because of the hook effect in LFDs packaged in bulk from the government supplier. Serial dilution as recommended in a protocol developed for the analysis of samples *(9)* resolved this problem. When sample throughput must be increased greatly (e.g., under surge conditions), samples may be pooled.

Chain specificity. Tetracore LFDs displayed a preference for the ricin B chain, and LFDs from the government supplier recognized the A chain. This complementation provided a useful confirmation in the screening of samples. The 10% cross-reaction with the other chain that occurred with LFDs from both suppliers may be indicative of either contamination by the B chain in the commercially purchased A chain (and visa versa) or inclusion of the hinge region and therefore both chains in the epitope. Further experimentation is necessary to resolve this question.

Hook effect. A hook effect in the response of LFDs occurs when the amount of antigen in a sample overwhelms the amount of detector antibody present in the LFD. The resultant free antigen competes with the antigen-detector antibody complex for the capture antibody. This competition results in a decrease in the response and under extreme conditions produces false-negative results. The onset of a hook effect is also a function of the affinity constant (K_d) of the detector antibody. Hook effects cannot occur in ELISAs because of the inclusion of a wash step following sample incubation. Instead, excess antigen (saturation) in an ELISA results in a plateau (ceiling) in the response curve.

False-negative results due to the hook effect with the government-supplied LFDs at high concentrations of ricin differed among lots. In contrast, the LFDs supplied by Tetracore never yielded false-negative results, although a quantitative decrease in the response has been repeatedly observed at high ricin concentrations (the hook effect). The incorporation of a serial dilution step in the sample protocol eliminated such potential errors in the processing of samples.

Assay design. The detection of low-molecular-weight agents poses a special problem for immunodiagnostic devices. Often the only solution possible for agents that do not possess two nonoverlapping epitopes is the use of a competitive assay. The key to the success of a competitive assay is reproducibility in the maximum signal measured that is representative of binding of the labeled agent without any competitors present. Any components present in the sample that interfere with the binding of the labeled agent would decrease the measured signal and produce false-positive results. One approach to minimizing this background signal in ELISAs is to wash the wells thoroughly to remove any non–antibody-antigen interactions. Removal is often facilitated by vigorous washing and inclusion of a detergent in the wash buffer. Another approach is to exploit the difference in the affinity of the nonspecific binding components causing the background signal and the affinity of the agent to the antibody. By diluting the sample such that the concentrations of the nonspecific binding components are at or below their K_d while the concentration of the agent is greater than its K_d, the assay should be effective. The flexibility to dilute the sample is in part limited by the limit of detection of the assay and the concentration that poses a health and safety concern. Both amanitin and T-2 toxin have sufficiently high oral LD_{50} values that the samples can be diluted without increasing the risk to the consumer.

In designing protocols for the detection of amanitin and T-2 toxin in food, the inclusion of a threshold response helped obviate the problem of an elevated background signal in the competitive ELISAs employed. Thresholds of 0.8 and 0.2 ppm for amanitin and T-2 toxin, respectively, avoided the background problems but were low enough to allow detection of these select agents at concentrations below those associated with serious health effects.

Commercial immunodiagnostic devices for the detection of ricin, amanitin, and T-2 toxin were successfully applied to the analysis of various foods. In all cases, the ricin, amanitin, and T-2 toxin were detected at concentrations lower than those associated with health risks. The high oral LD_{50} values of these select agents enabled the refinement of the assays such that nonspecific binding, hook effects, and other problems typical of immunoassay analysis of complex mixtures were inconsequential.

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