OXFORD

Journal of AOAC INTERNATIONAL, 104(1), 2021, 39–48

doi: 10.1093/jaoacint/qsaa113
Advance Access Publication Date: 25 August 2020
Article

SPECIAL GUEST EDITOR SECTION

Overview of Portable Assays for the Detection of Mycotoxins, Allergens, and Sanitation Monitoring

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Abstract

Background: Many food recalls are related to the presence of undeclared allergens and microorganisms in food products. To reduce these occurrences, portable diagnostic assay kits are available to quantitate mycotoxins, to detect allergens and gluten in foods and on environmental surfaces, and for sanitation monitoring.
Objective: This article reviews diagnostic kits that can detect sources of contamination in food and ingredients as well as on surfaces and clean-in-place rinses.
Method: Mycotoxins and gluten were detected using lateral flow diagnostic assays. Sanitation monitoring of surfaces was completed using a chemiluminescent assay to detect adenosine 5′-triphosphate disodium salt hydrate (ATP) and another assay to detect protein.
Results: Gluten was detected at 10 ppm in spiked commodities and on wet and dry surfaces at 2.5 μg/100cm^2. Deoxynivalenol was quantitated in dry distillers grains plus solubles and mean results were within two SDs of those determined by HPLC. The chemiluminescent assay had an LOD of 6 fmol of ATP and was able to detect a 1:10 000 dilution of orange juice from surfaces. The protein assay detected 5 μg of bovine serum albumin (BSA) directly applied to the sampler, 100 μg of BSA on surfaces, and detected 1:10 dilutions of Greek yogurt and raw beef from surfaces.
Conclusions: Portable diagnostic kits evaluated in this work provided accurate, rapid, and sensitive results for detection of mycotoxins, gluten, proteins, and ATP. These methods can be used in facilities with minimal training and provide results that are important to ensure food safety.
Highlights: Portable methods to detect gluten, mycotoxins, proteins, and ATP are presented.

Many food recalls occur due to the presence of undeclared allergens, chemical contaminants, and microorganisms. Allergens that are commonly involved in recalls are peanut, soy, gluten (wheat), milk, egg, tree nut, crustacean shellfish, and fish (1). While exposure to most of these allergens in allergic individuals produces similar side effects, such as hives, shortness of breath, allergic rhinitis, and swelling, symptoms of gluten intolerance or celiac disease also significantly impact the small intestine. Celiac disease affects one in 133 Americans and is an autoimmune disease that causes damage to the small intestine upon ingestion of gluten (2). Allergenicity has in some cases been linked to proteins or peptide residues in those commodities (3).

Received: 17 March 2020; **Revised:** 16 July 2020; **Accepted:** 16 August 2020

The identification of specific proteins responsible for allergenicity has enabled development of molecular assays which detect the DNA encoding the protein or peptides. Even when the specific protein or peptide responsible for the allergenic response is not known, proteins or peptides specific to the commodities can be used to develop immunological based assays for the allergen. Common methods used to detect allergens include PCR, ELISA, and lateral flow technologies. Of these methods, lateral flow devices (LFDs) have proved to be the most rapid and portable (4). These devices can also be easily performed in the field with little training and do not require any equipment. These features make LFDs ideal for environmental and sanitation testing where qualitative results are required in minutes and laboratory equipment is not commonly available.

In addition to allergens, chemical contaminants such as mycotoxins are also a significant issue for food safety (5–8). Mycotoxins can occur in foods when cereal grains, nuts, and other biomaterials are infected with *Aspergillus*, *Penicillium*, or *Fusarium* species which produce mycotoxins. There are over 200 toxigenic strains of *Fusarium* that produce various mycotoxins including T2-toxin, deoxynivalenol (DON) and nivalenol, fumonisin, and zearalenone (9). There are also *Aspergillus* strains that can produce aflatoxin and ochratoxin and *Penicilium* strains that can produce patulin and other toxins (9). Fortunately, assays are available to detect mycotoxins in grains, processed foods, and ingredients (10–14). Quantitative HPLC, LC/MS, and ELISA assays have been validated for quantitation of mycotoxins in a variety of commodities (13–15). Most HPLC and LC/MS assays are conducted in analytical laboratories and require expensive equipment and skilled operators to collect and analyze the data. Quantitative ELISAs can be performed in non-laboratory settings, but are not easily performed in a remote location, whereas lateral flow immunochromatographic assays can be performed in the field with minimal training. Several of these portable and rapid LFD mycotoxin assays have been developed which permit the use of these assays in non-laboratory settings (11, 16–18).

Adenosine 5′-triphosphate disodium salt hydrate (ATP) is an energy source for cellular metabolism and is found in all living organisms. Very sensitive assays are available to quantitate the amount of ATP in samples, so systems based on detection of ATP have been widely adopted as general sanitation assays (19–25). Several of these assays detect residual ATP based on the luciferase catalyzed reaction of Mg·ATP and luciferin to produce light which is detected by a photocell. Chemiluminescence based methods can detect femtomole quantities of ATP using relatively inexpensive detectors. Colorimetric assays are also available to detect residual protein which can be used for allergen and sanitation monitoring. In general, colorimetric assays for protein detection are less sensitive than chemiluminescence based ATP or immunological assays, but the colorimetric assays don't require additional equipment to obtain results. This article discusses portable diagnostic assays and results for detection of allergens and contaminants in food, ingredients, and on environmental surfaces.

METHOD

Reveal® 3D for Gluten Lateral Flow Assay

Reveal® 3D for Gluten (PN 8505, Neogen Corp., Lansing, MI) is a rapid lateral flow assay that detects the presence of gluten residues in foods, rinses, and surfaces. The assay can detect down to 5 ppm gluten in rinse samples and foods and 2.5 μg/100cm^2 on surfaces in 5 min. The test works by applying sample extract to the sample pad of the device, where it is wicked through the reagent zone where any gluten in the sample binds to anti-gluten antibodies bound to colloidal gold. Once the extract reaches the nitrocellulose, anti-gluten antibodies on the test line bind the gluten bound to the antibody-gold complex, forming a visible test line. The overload line captures any unbound antibody-gold molecules, also forming a visible line. As the concentration of gluten in the sample increases, the test line intensity will increase while the overload line intensity will decrease and eventually disappear. Validation of this assay was completed through evaluation of each sampling method and through verification of the assay's detection of gluten.

Probability of detection (POD)

The likelihood of the assay to detect the presence of gluten at various levels was challenged in a POD study. For this study, three operators screened two different lots of devices over the course of two days. Each operator prepared samples by extracting gluten in ethanol and diluting to the listed concentrations. The samples were then extracted via the kit insert for CIP rinses by adding 0.25 mL of each sample to the extraction buffer provided in the kit and hand shaking for 1 min.

CIP rinse verification

The performance of the kit was evaluated by spiking CIP cleaner at working strength with known concentrations of gluten. This study was conducted by one operator on one lot over the course of one day and evaluated five different samples. The spiked samples were then extracted per the kit insert for CIP samples by adding 0.25 mL of sample to the kit extraction buffer and hand shaking for 1 min.

Swab recovery verification

To evaluate the sensitivity of the swab sampling method, a 10 × 10 cm stainless steel surface was spiked with known amounts of gluten. For the dry surface recovery study, the sample was completely dried prior to testing. The wet surface sampling occurred while the surface still retained moisture. The surface was then swabbed according to test instructions. The swab was placed in the extraction buffer provided with the kit and shaken for 1 min. This study was performed by one operator using five different samples and tested on one lot of Reveal® 3D for Gluten over the course of one day.

Food extraction verification

Both sensitivity and selectivity were examined using the food extraction method. Sensitivity was evaluated by spiking rice flour with gluten at known concentrations and extracting using the gluten food extraction buffer kit. For this method, 2 g of sample is added to 20 mL of food extraction buffer and hand shaken for 1 min. Once any particulate has settled, 0.8 mL of the sample is added to the kit extraction buffer and hand shaken for another minute. Selectivity of the assay was examined by spiking 10 different commodities with known concentrations of gluten and extracting in the same method outlined for food extraction above.

Once the sample has been prepared, the assay is run by dipping the lateral flow device in the extracted solution until the flow reaches the viewing window. The device can then be placed on a flat surface for the remainder of the 5 min development time. After 5 min, the device can be visually read and interpreted as positive or negative.

Quantitation of Mycotoxins by Lateral Flow Immunochromatographic Assay

Reveal® Q+ MAX for DON (PN 8388, Neogen Corp.) is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The extract is wicked through a reagent zone, which contains antibodies specific for DON conjugated to colloidal gold nanoparticles. If DON is present, it will be captured by the nanoparticle-antibody complex. The DON-antibody-particle complex then is wicked onto a membrane, which contains a zone of DON conjugated to a protein carrier. This zone captures any un-complexed DON antibody, allowing the particles to concentrate and form a visible line. As the level of DON in a sample increases, free DON will complex with the antibody-gold particles. This allows less antibody-gold to be captured in the test zone. Therefore, as the concentration of DON in the sample increases, the test line density decreases. Algorithms programmed into the AccuScan® readers convert these line densities into a quantitative result displayed in parts per million (ppm). The membrane also contains a control zone where an immune complex present in the reagent zone is captured by an antibody, forming a visible line. The control line will always form regardless of the presence of DON, ensuring the strip is functioning properly.

Ten grams of each dry distillers' grain with solubles (DDGS) sample were weighed into sample extraction cups. The contents of one MAX 1 (PN 8089, Neogen Corp.) aqueous extraction packet were added into each sample extraction cup. Then, 50 mL of deionized water was added to each of the sample cups and they were vigorously shaken on a mechanical stirrer for 3 min. The samples were settled for 10 min and then filtered through a filter syringe collecting 3 mL of sample filtrates into sample collection tubes. If necessary, the pH of the samples was adjusted to pH 7.0 by adding drops of 5–7 N NaOH.

Sample diluent (1000 μL) was added to a sample dilution cup and 100 μL of sample extract was then added to the sample diluent. This was mixed by pipetting up and down five times. Diluted sample extract (100 μL) was transferred into a new sample cup and a Reveal® Q+ MAX for DON (PN 8388, Neogen Corp.) test strip with the sample end down was added into the sample cup and a set timer for 3 min. It was ensured the test strip was in contact with liquid and began to wick. After 3 min, the strip was removed from the sample cup and read in either an AccuScan Gold or Pro reader.

Quantitation of Mycotoxins by HPLC Assay

The analytical method used for DON is based on the method described by MacDonald (26). Twenty-five grams of sample were extracted using 200 ml of water. The mixture was blended for 2 min before being filtered through a glass fiber filter paper. The pH was tested and, if necessary, was adjusted to between pH 6 and 8. Five milliliters of the extract were applied to an immunoaffinity column (PN 8340, Neocolumn, Neogen Corp.), which was then washed with 12.5 ml of deionized DI water. The samples were eluted with 1.5 ml of methanol and were then evaporated to dryness under a stream of nitrogen. After the solvent was removed, the samples were reconstituted in 1 ml of 90:5:5 H_2O: ACN: MeOH. One hundred microliters of sample was injected onto a Waters Sunfire C18 5 μm column which was held at 40°C. The mobile phase was 1.5 ml/min of 90:5:5 H_2O: ACN: MeOH and detection was done by UV at 218 nm.

Portable Surface Sanitation Assay

ATP is an energy source for cellular metabolism and is found in all living cells. Since very sensitive assays are available to quantitate the amount of ATP in samples, systems based on detection of ATP have been widely adopted as general sanitation assays. AccuPoint® Advanced (PN 9905, Neogen Corp.) is a general sanitation assay that detects ATP using the chemiluminescent reaction that occurs when ATP and luciferin bind to luciferase in the presence of magnesium to produce light. The amount of light produced is a function of the amount of ATP available for the reaction. The chemiluminescent reaction uses samplers and a portable luminescence reader for general sanitation monitoring. Samplers were equilibrated to room temperature prior to testing. Analytical standard grade ATP was purchased from Sigma Chemical Co (St. Louis, MO)., part # FLAAS. A stock solution of 50.0 nM ATP in 50 mM tricine buffer, pH 7.75 was prepared and the concentration verified by ultraviolet spectrophotometry using an extinction coefficient at 259 nm of 15.4×10^3 L/M·cm (27). Dilutions in ultrapure water (resistivity of at least 18.2 MΩ•cm) were prepared at 5.00, 1.25, and 0.625 nM.

Evaluations of the sanitation system included (1) addition of ATP standard solutions directly to sample swabs, (2) recovery of ATP deposited over a 10 × 10 cm stainless steel surface and (3) recovery of orange juice dilutions from a 10 ×10 cm stainless steel surface.

Evaluation of direct addition of ATP standard solution to samplers

For direct evaluation of ATP standards in 50 mM tricine buffer, pH 7.75, ATP was applied directly to the swabs by carefully pipetting 20 μL of the ATP standard onto a sample swab or pad of the sampler. Twenty microliters of the 5.00, 1.25, and 0.625 nM solutions of ATP or sterile water resulted in the following femtomoles of ATP on the sample pad or swab; 100, 25.0, 12.5, and 0 fmol, respectively. Immediately following addition of the ATP standard to the sample pad or swab, they were placed in the sampler, activated, shaken for 3 s, and read on the luminescence reader. The results were plotted as relative luminescence units (RLU) versus known ATP concentration and s_r versus RLU. A linear trend line of the standard error of repeatability s_r versus RLU plot was used to determine the LOD with:

$$\text{LOD} = \bar{x}_o + 3.3(s_b)/1\text{–}1.65m$$

where $\bar{x}_o$ = the mean of the background value, s_b = the plot intercept, and m = the slope. The resulting RLU LOD was inserted as the y-value in the RLU versus ATP curve to determine LOD in fmol ATP.

Determining recovery of ATP from stainless steel surfaces

For determination of surface recovery, a stainless-steel plate with sixteen 10 × 10 cm squares was used. To avoid uncontrolled contamination, testing was conducted in a laminar flow hood equipped with a UV lamp. Prior to each round of testing, the plates and all required cleaning supplies were sterilized under the UV lamp for 20 min. The plates were then cleaned with isopropyl alcohol, wiped off, and cleaned again with a 10% Contrad 70 solution in water. Following this, the plates were thoroughly rinsed with sterile water and wiped dry with Chemwipes.

Recovery of ATP from 10 × 10 cm stainless steel surfaces was determined by using 20 μL of ATP solution that produced a

response of 1000 ± 50 RLU when added directly to the sampler and read using an AccuPoint Advanced reader. To prepare the ATP solution required, a 50 nM ATP solution (mass of 1000 femtomoles in a 20 μL aliquot) was diluted 3.4 fold producing a solution with 300 fmol of ATP in a 20 μL aliquot. For ATP recovery testing, 20 μL of that solution was evenly spread over the stainless steel surface and air dried for 1 hr at 22–25°C. Direct addition of 20 μL of the same solution containing 300 fmol of ATP to the sample pad of a surface sampler resulted in a response of 1000 ± 50 RLU on the luminometer. The process was repeated three times and the mean RLU calculated. That mean was the response for 100% recovery of ATP since ATP was directly added to the sample pad with no loss in signal due to surface sampling. The RLU was then measured for the same mass of ATP recovered from the stainless steel surface. The surface recovery experiment was repeated ten times to determine the mean response. The mean response was divided by 1000 which resulted in the percentage of recovered ATP.

For detection of ATP in commodities on surfaces, orange juice solutions were prepared with dilutions in sterile water

Commodity testing with orange juice was completed to determine recovery from a stainless steel surface and LOD. For this evaluation, a 1000 fmol ATP standard was diluted in a 1:8 ratio in both orange juice and milk. From there, the commodities were diluted using sterile water to levels of 1:20, 1:100, 1:1000, and 1:10 000 for orange juice as well as 1:10, 1:100, 1:1000, and 1:10 000 for milk. Samples of each dilution level were prepared by dispensing 50 μL of a given dilution level as evenly as possible across the surface of each plate and allowing the samples to dry for one hour before sampling according to the prescribed method for each brand of sampler.

Protein Detection on Surfaces

Protein was detected using AccuClean® Advanced (PN 9960, Neogen Corp.) a portable assay system used to detect proteins. To determine protein detection limit and linearity of the assay, a 10.0 mg/mL stock solution of bovine serum albumin (BSA) in water was prepared by dissolving 120 mg. of BSA in 12.0 mL of sterile filtered purified water. The stock BSA solution was diluted to 5.00, 2.50, 1.00, 0.50, and 0.25 mg/mL. To determine the detection limit of the assay for BSA, 20 μL of each of the solutions was evaluated with five replicates and two lots of samplers by three operators on two days. Blank conditions used 20 μL of ultrapure water added directly to samplers. To collect data using the protein assay, the sample handle was removed from the sampler. Then, 20 μL of each BSA standard solution or blank was pipetted directly onto the sample pad. The sample handle was inserted into the sampler and the handle was fully depressed puncturing the foil seal at the bottom of the sampler. The solution was swirled in the sampler for 10 s. The results were interpreted by examining the color of the solution where formation of a gray or blue color indicated protein was detected.

To evaluate detection of proteins from food surfaces, a 1:10 slurry of Greek yogurt in water and a 1:10 slurry of raw beef in water were prepared by stomaching 5 g of the food sample with 45 mL sterile Type 1 water for 30 min until thoroughly blended. Fifty microliters of slurry was spread over 10 × 10 cm clean stainless steel and plastic surfaces and allowed to dry for 1 h. Fifty microliters of sterile water aliquots were also tested as a negative control. The surfaces were sampled by removing a sample handle from the sampler and swabbing the surface. The sample handle was then inserted into the sampler and the handle fully depressed puncturing the foil seal at the bottom of the sampler. The solution was swirled in the sampler for 10 seconds. The results were interpreted by determining the color of the solution with formation of a gray or blue color indicating protein was detected.

Results and Discussion

Analysis of Gluten using Reveal® 3D Lateral Flow Technology

POD

Figure 1 outlines the results for the POD testing. The number of replicates tested is outlined in Table 1. The results indicate that fractional positives can be achieved at levels below the claimed LOD of 5 ppm. Detection rate at 5 ppm gluten is 100% and no false positives were observed.

CIP rinse testing

A working strength (25%) ammonium-based cleaner was used for this study in which five samples were tested for each spike

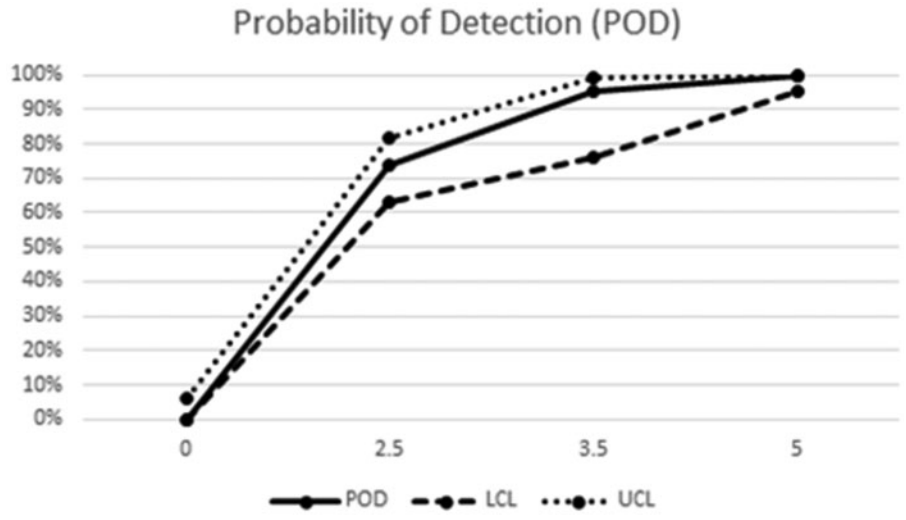

Figure 1. Probability of detecting gluten in CIP rinse using Reveal® 3D for Gluten lateral flow assay (upper and lower confidence intervals are also shown).

level. Table 2 depicts the test results. The line intensity of the assay is measured using a score card with line intensity values ranging from 0–5, with five being the most intense. The average results for the test line intensity are included here to provide a representation of how the assay functions, as described in the introduction. Results indicate 100% detection in ammonium based cleaner at and below the claimed LOD of 5 ppm.

Surface swab recovery

To verify recovery on surfaces, ten stainless steel squares were contaminated with various levels of gluten. Five of these surfaces were wet when the swab sample was taken while the remaining five were dried completely before testing. Table 3 depicts the results of this testing. One hundred percent positive results were observed at 2.5 μg/100cm^2 and the average test line intensity for each level was reported. While the qualitative results were identical for both wet and dry surfaces, the average intensities of the test line were greater when wet surfaces were tested.

Food extraction and recovery

Sensitivity testing was completed by spiking rice flour with known amounts of gluten and extracting using the food extraction method. The results and number of samples tested for this study are outlined in Table 4. Results indicate that 100% detection is achieved in rice flour at the claimed LOD of 5 ppm while results at 2.5 ppm gluten are negative. Selectivity testing was completed by extracting ten different matrices, both un-spiked and spiked with gluten at 10 ppm. One sample replicate was performed for each data point described in Table 5. These results indicate the assay does not have cross-reactivity or matrix interference from the commodities tested.

Table 1. POD with 95% upper and lower confidence levels of gluten residues in CIP rinse using Reveal® 3D for Gluten lateral flow assay

Concentration, ppm gluten	No.	Positive results	POD, %	LCL, %[a]	UCL, %[b]
0	60	0	0	0	6
2.5	80	59	74	63	82
3.5	20	19	95	76	99
5	80	80	100	95	100

[a] LCL = Lower confidence levels.
[b] UCL = Upper confidence levels.

Table 2. Recovery of gluten residues in working strength CIP cleaner

Concentration, ppm gluten	Positive results, %	Average line intensity (Scale of 0 to 5)
0	0	0
2.5	100	1
5	100	2.1
10	100	3.1

Determination of DON in DDGS by Lateral Flow and HPLC

Since DDGS is a byproduct of ethanol production from corn, it can be contaminated by heat stable mycotoxins if the corn used was infected by toxigenic fungus. Often, DDGS is used as a feed additive, therefore, residual mycotoxin contamination is a concern for animal safety. Because of this risk, the level of residual mycotoxin in the DDGS should be determined. To evaluate the performance of the LFD assay, samples of DDGS from several production lots were split and evaluated for residual DON by Reveal® Q+ for DON and by a quantitative HPLC method. Table 6 provides the mean and SD of the quantitative results by each method for the determination of DON in those samples. The lateral flow results are the mean of three test replicates from two separate sample extracts. The HPLC results are the mean results from several analytical laboratories conducted on the same samples split between laboratories. The DON concentrations ranged from about 0.3 ppm for sample DON-3 to a high of about

Table 4. Recovery of gluten from spiked rice flour using food extraction method for the Reveal® 3D for Gluten lateral flow assay

Concentration, ppm gluten	No.	Positive results, %
0	25	0
2.5	5	0
5	25	100
10	5	100

Table 5. Selectivity data demonstrating results of an un-spiked commodity in comparison to the same commodity spiked at 10 ppm gluten

Commodity	0 ppm	10 ppm
Brown rice flour	–	+
Sweet rice flour	–	+
Sorghum flour	–	+
Spices	–	+
Coconut flour	–	+
Chestnut flour	–	+
Oats	–	+
Milk powder	–	+
Quinoa flour	–	+
Tea	–	+

Table 3. Recovery of gluten residues on wet and dry surfaces using surface swab recovery method

	Wet surface		Dry surface	
Concentration, ppm gluten	Positive results, %	Average test line intensity	Positive results, %	Average test line intensity (Scale of 0 to 5)
0	0	0	0	0
2.5	100	2.6	100	2.1
5	100	3	100	2.4
10	100	4	100	2.8
15	100	5	100	3.3

Table 6. Summary of mean DON results obtained by LFDs and HPLC for several DDGS samples

Sample	Mean LFD, ppm	LFD SD	Mean HPLC, ppm	HPLC SD	Difference LFD vs HPLC, %
DON-3	<0.3	NM	0.3	0.2	NM[1]
DON-5	11.8	0.8	13.8	2.5	−17
DON-9	6.4	0.2	7.1	1.3	−11
DON-1	12.3	1.2	12.2	1.8	1
DON-2	10.7	0.6	10.8	1.5	−2
DON-12	12.7	0.6	12.7	2.5	0
DON-16	6.7	0.7	7.0	1.2	−4
DON-20	12.7	0.6	12.1	2.0	5
DON-6	10.5	0.3	10.4	1.0	1
DON-7	7.7	0.2	6.8	0.8	12
DON-15	4.2	0.2	4.0	0.6	5
DON-4	11.8	0.4	13.0	1.6	−10
DON-8	10.8	0.5	10.7	1.3	1
DON-11	11.5	0.5	12.0	1.2	−4
DON-10	4.4	0.1	4.2	0.6	4
DON-13	11.1	0.5	12.8	0.9	−16
DON-14	9.5	0.6	11.5	1.2	−21

[1] NM - not meaningful

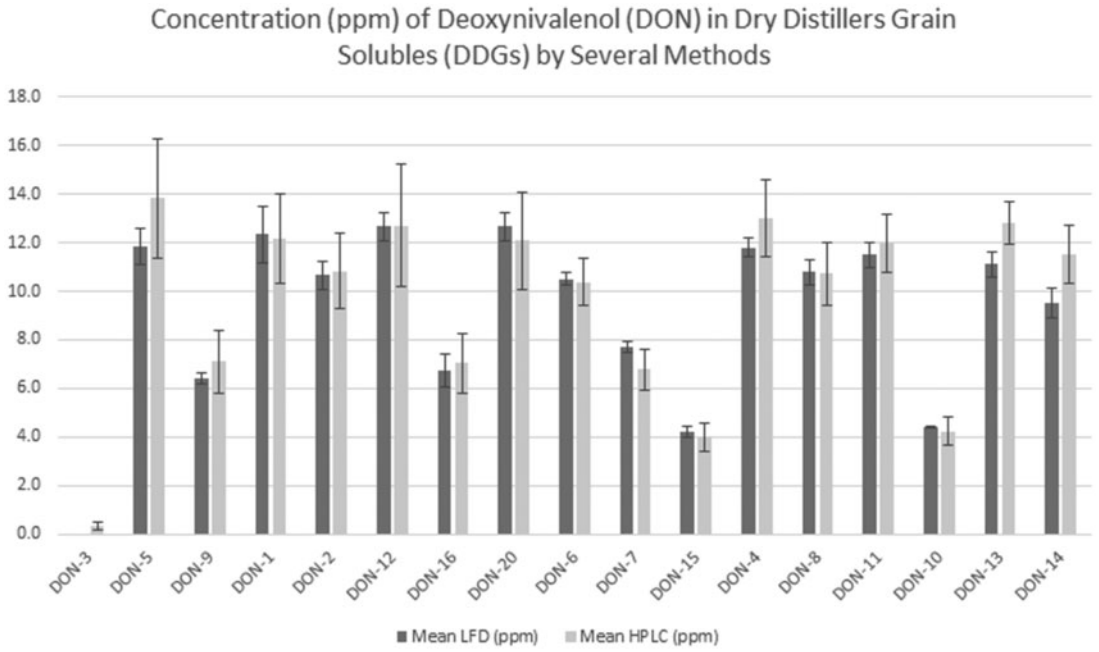

Figure 2. Concentration (ppm) of DON in DDGS by LFD and HPLC.

14 ppm for sample DON-5. Overall, there was good agreement between LFD and HPLC results. The mean LFD results for 14 of the 17 DDGS samples were within 12% of the mean HPLC values. Mean results for 3 of the 17 DDGS samples were biased low for samples DON-5 (–17%), DON-13 (–16%), and DON-14 (–21%) compared to the mean HPLC results but were still within 2 ppm. SDs for the LFD results were <0.8 ppm, except for one sample where the SD was 1.2 ppm. In general, SDs for the mean HPLC results were greater than LFD but HPLC test results includes interlaboratory variability.

A bar chart depiction of results is also shown in Figure 2. The error bars in Figure 2 are ± 1 SD. Except for sample DON-13 and DON-14, all the LFD results were within 1 SD of the interlaboratory HPLC mean indicating there was good agreement between LFD and HPLC results for each sample. Sample DON-3 had no detectable DON in the sample by LFD and only 0.3 ppm by HPLC. In addition, there was good precision of the results as indicated by the relatively low SDs for replicate measurements.

General Sanitation Monitoring

Direct measurement of ATP standards

The general sanitation assay described in the *METHOD* section was used which detects ATP through the chemiluminescent reaction that occurs when ATP and luciferin bind to luciferase in

Table 7. Surface sanitation sampler response for ATP standards pipetted directly onto sampler swabs

	RLU at each [ATP] fmol			
Sample No.	0 fmol of ATP	12.5 fmol of ATP	25 fmol of ATP	100 fmol of ATP
1	0	54	156	781
2	0	62	163	722
3	0	47	163	777
4	0	46	153	763
5	0	40	192	792
6	0	46	179	783
7	0	45	147	716
8	0	61	172	779
9	0	48	159	694
10	0	54	128	802
11	0	43	157	662
12	0	54	181	771
13	0	60	128	655
14	0	54	145	725
15	0	33	184	742
16	0	42	168	813
17	0	45	165	834
18	0	24	179	699
19	0	45	161	809
20	0	28	187	757
21	0	59	150	728
22	0	44	152	856
23	0	46	170	806
24	0	40	160	759
25	0	39	157	821
Average	0	46	162	762
SD	0	10	16	52
[1]CV, %		20.8	10.1	6.8

[1] CV=SD/Average x 100

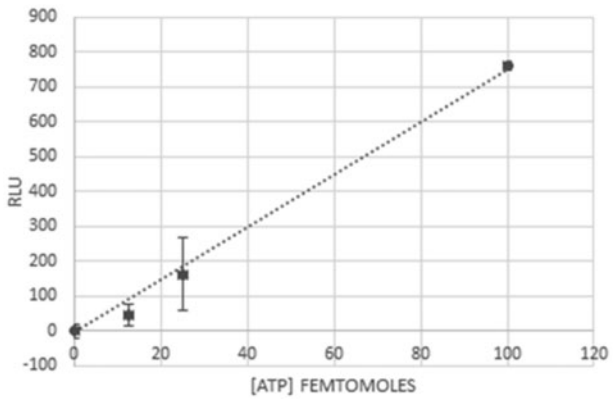

Figure 3. RLU versus ATP femtomoles.

the presence of magnesium to produce light. The amount of light produced is a function of the amount of ATP available for the reaction. Table 7 lists the RLU obtained when standard solutions of ATP were added directly to the sampling pad of samplers. For each concentration of ATP, 25 different trials were performed and the mean and SDs for those trials are provided in the table. Luminescence response is relative due to several factors including reader optics, detector type (photodiode or photomultiplier), detector gain setting, and luminescence reaction chemistry, so the absolute magnitude of response can differ between different manufacturers of sanitation assays. Mean assay response and SD for 0, 12.5, 25.0, and 100.0 fmol of ATP was 0, 46 ± 10 RLU, 162 ± 16 RLU, and 762 ± 52 RLU, respectively. Assay response was linear as shown in Figure 3 with a correlation coefficient squared (R^2) of 0.99 and a slope of 7.5 RLU/fmol of ATP. The LOD determined by the equation as described in the *METHOD* section was 6.2 fmol of ATP.

Recovery of ATP from a stainless steel surface. In addition to sensitivity and precision, the ability of the samplers to recover ATP from stainless steel surfaces is an important metric to evaluate the ability of samplers to recover organic material from that surface. The first row of data in Table 8 lists the mean RLU and SD from three samplers with 300 fmol of ATP pipetted directly onto the swabs of the samplers. The table provides assay results for the chemiluminescent assay described in the *METHOD* section and four other types of ATP sanitation samplers that use woven polymer swabs for sampling. Row 2 lists the mean RLU and SD from 10 samplers where the same mass of ATP was deposited over a previously cleaned 10 × 10 cm stainless steel surface, dried, and then sampled in the manufacturer's prescribed manner using swabs for each brand. Row 3 lists the percentage of ATP recovered from the surface compared to the RLU measured when the same mass of ATP was pipetted directly onto the swab. Surface recovery of ATP for the method used in this study was 5 to 10 times greater than recoveries obtained using sanitation assays that used "Q-tip" type swabs. The benefit of greater recovery of ATP from the surface was apparent using a flat sampling pad versus the "Q-tip" swabs. In addition, precision of RLU measured for ATP recovered from the surface was better using the flat sampling pads. Coefficients of variation determined from the RLU and SD for ATP recovered from the surface were 17.5% for the assay used in this work and 43.3, 51.2, 72.1, and 94.0% for the other swabs.

Recovery of dried orange juice from a 4 × 4" stainless steel surface. A situation likely encountered for sanitation devices is recovery of food residues from stainless steel surfaces. This was simulated in a controlled experiment by depositing the same fixed aliquot of orange juice over 10 × 10 cm stainless steel surfaces and recovering the material using various brands of sanitation samplers following the manufacturer's instructions. Table 9 lists the results for each of the brands at several dilutions of orange juice in sterile water that were dried on the 10 × 10 cm stainless steel surfaces. Consistent with previous results, precision of RLU measured using the flat sampling pad for the sanitation assay described in the *METHOD* section was better than the other brands of sanitation samplers at all dilutions of orange juice including the largest dilution of 1:10 000.

Protein Determination by Colorimetric Assay

Results for detection of BSA protein at several concentrations pipetted directly onto two different lots of samplers are provided in Table 10. These results are from one operator on one day of testing. Ultrapure water was added to samplers as the blank. Results for the blank are listed under the column for 0 μg of BSA. There were no false positive results obtained for the blank samples by this operator for either lot of samplers. Starting with 5 μg of BSA added to the samplers, each test provided a positive result for protein detection as indicated by gray color formation at 5 μg BSA up to 20 μg of BSA. Results on

Table 8. Mean response for recovery of 300 fmol of ATP deposited and dried on a surface relative to direct addition to the sampler

Swab Type	Flat sampling pad	Swab brand 2	Swab brand 3	Swab brand 4	Swab brand 5
Mean RLU and SD direct addition to sampler	1038.0 ± 86.1	1461.0 ± 63.0	624.3 ± 65.3	59002.3 ± 7590.68	1472.3 ± 432.3
Mean RLU and SD recovered from surface	339.5 ± 59.3	49.8 ± 21.5	21.5 ± 11.0	1793.7 ± 1294.1	98.6 ± 92.7
Recovery, %	32.71	3.41	3.44	3.04	6.70

Table 9. Recovery of dried orange juice from 4 ×4" stainless steel surfaces

Orange juice dilution	Assay described in methods	Brand 2	Brand 3	Brand 4	Brand 5
1:20, RLU	46728.9	10294.9	3879.1	218089.2	
1:20, SD	7065.5	6160.1	1845.6	53281.4	
1:20, CV %	15.1	59.8	47.6	24.4	
1:100, RLU	9995.1	2544.2	593.2	101990.7	795.8
1:100, SD	2123.8	1112.0	359.2	58153.6	482.4
1:100, CV %	21.2	43.7	60.6	57.0	60.6
1:1000, RLU	874.3	447.1	147.4	54438.2	123.8
1:1000, SD	137.9	195.5	55.1	19605.1	105.7
1:1000, CV %	15.8	43.7	37.4	36.0	85.3
1:10 000, RLU	92.3	122.2	11.6	11118.3	15.3
1:10 000, SD	22.8	64.6	5.9	4400.8	11.4
1:10 000, CV %	24.7	52.8	51.0	39.6	74.6

[1] CV=SD/RLU x 100

Table 10. Detection of different masses of bovine serum albumin by colorimetric assay

Lot Number	Sample	0 μg	5 μg	10 μg	20 μg	50 μg	100 μg	200 μg
Lot 1	1	Brown	Gray	Gray	Gray	Blue	Blue	Blue
	2	Brown	Gray	Gray	Gray	Blue	Blue	Blue
	3	Brown	Gray	Gray	Gray	Blue	Blue	Blue
	4	Brown	Gray	Gray	Gray	Blue	Blue	Blue
	5	Brown	Gray	Gray	Gray	Blue	Blue	Blue
Lot 2	1	Brown	Gray	Gray	Blue	Blue	Blue	Blue
	2	Brown	Gray	Gray	Blue	Blue	Blue	Blue
	3	Brown	Gray	Gray	Blue	Blue	Blue	Blue
	4	Brown	Gray	Gray	Blue	Blue	Blue	Blue
	5	Brown	Gray	Gray	Blue	Blue	Blue	Blue

Table 11. Overall results for colorimetric assay detection of BSA directly added to samplers by three operators on two days with two lots of samplers

Lot Number	POD for direct addition of BSA on samplers (listed by mass of BSA)						
	0 μg	5 μg	10 μg	20 μg	50 μg	100 μg	200 μg
Lot 1, %	10.0	100.0	100.0	100.0	96.7	100.0	100.0
Lot 2, %	3.3	100.0	100.0	100.0	100.0	100.0	100.0
Overall POD, %	6.7	100.0	100.0	100.0	98.3	100.0	100.0

the first lot produced a gray color and the second lot produced a blue color in the samplers. Above 50 μg of BSA added, all results were blue or strong positive for protein detection.

On both days of testing by three operators, there was no change in color for the blanks except for results from one operator on the first day. For that operator, 3 of 30 tests were incorrectly identified as positive for BSA using one lot of samplers and 1 of 30 tests incorrectly identified as positive using the second lot of samplers. After the first day of testing, the operator was more familiar with the color change expected for positive samples and did not observe any false positives on the second day of testing with either lot. Table 11 provides an

Table 12. Overall results for colorimetric assay detection of BSA from stainless steel surfaces by three operators on two days with two lots of samplers

Lot Number	POD for recovery of BSA from surface (listed by mass of BSA)				
	0 μg	100 μg	200 μg	400 μg	1000 μg
Lot 1, %	0.0	93.3	100.0	100.0	100.0
Lot 2, %	0.0	100.0	100.0	100.0	100.0
Overall POD, %	0.0	96.7	100.0	100.0	100.0

Table 13. Overall results for colorimetric assay detection of yogurt and beef from stainless steel and plastic (polyethylene) surfaces

POD for food recovery from surfaces						
Surface Type	Plastic			Steel		
Lot Number	[1]Neg, %	Yogurt, %	Beef, %	[1]Neg, %	Yogurt, %	Beef, %
Lot 1	0.0	100.0	86.7	0.0	96.7	100.0
Lot 2	0.0	100.0	90.0	0.0	100.0	96.7
Overall POD	0.0	100.0	88.3	0.0	98.3	98.3

[1]Neg- negative is a clean surface with no food product added.

overall summary of all results for all operators on both days of testing. Overall, PODs for detection of ≥5 μg of BSA were 100%, except for 1 of 60 samplers that did not produce a positive color change with 50 μg of BSA added which resulted in a POD of 98.3%. A 6.7% probability for a false positive detection of BSA was noted with the colorimetric assay. That was reduced once operators were familiar with the color change expected for samples containing protein.

Overall results from three operators on two days evaluating detection of BSA at several concentrations recovered from stainless steel surfaces using two different lots of samplers are provided in Table 12. For the blank using ultrapure water added to samplers there was no change in color for all operators on all days for all 60 blank samples. Overall, PODs for detection of ≥200 μg of BSA from stainless steel surfaces were 100%. Detection of 100 μg of BSA from stainless steel surfaces was 96.7% with 2 of 60 false negatives reported. One false negative occurred for each lot of samplers for recovery of 100 μg of BSA from stainless steel.

Overall results from three operators on two days evaluating detection of yogurt and beef recovered from stainless steel and plastic (polyethylene) surfaces using two different lots of samplers are provided in Table 13. For the blank, ultrapure water added to samplers, there was no change in color for all operators on all days for all 60 blank assays. Overall POD for detection of yogurt on plastic was 100%. For detection of yogurt on stainless steel surfaces the POD was 98.3% where 1 of 60 assays resulted in a false negative result. Detection of beef from plastic resulted in a POD of 88.3%, where there were 7 of 60 false negative results from one operator on one day. Detection of beef from stainless steel resulted in a POD of 98.3%, where there was 1 of 60 false negative results.

Conclusions

We demonstrated that the portable assays described here are reliable tools for detection of mycotoxins, gluten, proteins, and ATP. Lateral flow devices for analysis of mycotoxins and allergens are easy to use with minimal training required to obtain reliable results as are the samplers for protein and ATP assays that are described. Rapid assays providing evidence for proper cleaning and sanitation practices are of heightened interest.

Acknowledgments

The authors thank Danielle Delamarter, Evan Meister, and Kaelee Gonzalez for data collection using the protein detection assay and Doug MacRae for his portion of data collection using the ATP detection assay.

All work in this report was performed by employee's of Neogen Corp. and supported by Neogen Corp., Lansing MI.

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