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, 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 (CIP) rinses.

Methods

Mycotoxins and gluten were detected using lateral flow diagnostic (LFD) assays. Sanitation monitoring of surfaces was completed using a chemiluminescent assay to detect 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/100cm². Deoxynivalenol was quantitated in Dry Distillers Grains plus Solubles (DDGs) and mean results were within two standard deviations (SD) of those determined by high performance liquid chromatography (HPLC). The chemiluminescent assay had a limit of detection (LOD) of 6 femtomoles of ATP and was able to detect a 1:10,000 dilution of orange juice from surfaces. The protein assay detected 5 µg of 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 provided results that are important to ensure food safety.

Highlights

Portable methods to detect gluten, mycotoxins, proteins and ATP are presented.
Introduction

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 1 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). 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 allergic 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 polymerase chain reaction (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 and Fusarium species which produce mycotoxins. There are over 200 toxigenic strains of Fusarium that produce various mycotoxins including T2-toxin, deoxynivalenol (DON) and nivalenol, fumonisins, zearalenone (9). There are also Aspergillus strains that can produce aflatoxin and ochratoxin and Penicillium 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).

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.

Methods

Reveal® 3D 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/100cm² on surfaces in 5 minutes. 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.

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 minute.

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 one minute.

Swab Recovery Verification: To evaluate the sensitivity of the swab sampling method, a 10 cm x 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 minute. This study was performed by one
operator using five different samples and tested on one lot of Reveal® 3-D 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 minute. 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-minute development time. After 5 minutes, the device can be visually read and interpreted as positive or negative.

Quantitation of Mycotoxins by Lateral Flow Immunochromatographic Assay

Reveal® Q+ MAX for Deoxynivalenol (DON) (PN 8388, Neogen Corp., Lansing, MI) 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 Soluble (DDGs) sample were weighed into sample extraction cups. The contents of one MAX 1 (PN 8089, Neogen Corp., Lansing, MI) aqueous extraction packet were added into each sample extraction cup. Fifty mL of deionized water were added to each of the sample cups and they were vigorously shaken on a mechanical stirrer for 3 minutes. The samples were settled for 10 minutes 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 5 times. Diluted sample extract (100 μL) was transferred into a new sample cup and a Reveal Q+ MAX for DON (PN 8388, Neogen Corp., Lansing, MI) test strip with the sample end down was added into the sample cup and a set timer for 3 minutes. It was ensured the test strip was in contact with liquid and began to wick. After 3 minutes, 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 deoxynivalenol is based on the method described by MacDonald (26). Twenty-five grams of sample are extracted using 200 ml of water. The mixture was blended for 2 minutes 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., Lansing, MI), which was then washed with 12.5 ml of 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 H₂O:ACN:MeOH. One hundred microliters of sample was injected onto a Waters Sunfire C18 5µm column which was held at 40°C. The mobile phase was 1.5 ml/min of 90:5:5 H₂O: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., Lansing, MI) 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 adenosine 5’-triphosphate disodium salt hydrate (ATP) was purchased from Sigma Chemical Co., 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 x10³ L/M cm (27). Dilutions in ultrapure water (resistivity of at least 18.2 MΩ·cm) were prepared at 5.00 nM, 1.25 nM and 0.625 nM.

Evaluations of the sanitation system included a) addition of ATP standard solutions directly to sample swabs, b) recovery of ATP deposited over a 10 cm x 10 cm stainless steel surface and c) recovery of orange juice dilutions from a 10 cm x 10 cm stainless steel surface.
(a) 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 femtomoles, respectively. Immediately following addition of the ATP standard to the sample pad or swab, they were placed in the sampler, activated, shaken for 3 seconds and read on the luminescence reader. The results were plotted as 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:

\[ \text{LOD} = \bar{x}_o + 3.3(s_b) / 1 - 1.65m \]

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

(b) Determining Recovery of ATP from Stainless Steel Surfaces

For determination of surface recovery, a stainless steel plate with sixteen 10 cm x 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 twenty minutes. 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 cm x 10 cm stainless steel surfaces was determined by using 20 µL of ATP solution that produced a response of 1000±50 RLU (Relative Luminescence Units) 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 femtomoles 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 femtomoles 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.

(c) For detection of ATP in commodities on surfaces, orange juice solutions were prepared with 1:20, 1:100, 1:1,000 and 1:10,000 dilutions in sterile water.

Commodity testing with orange juice was completed to determine recovery from a stainless steel surface and limit of detection. For this evaluation, a 1000 femtomoles 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 1:10000 for orange juice as well as 1:10, 1:100, 1:1000 and 1:10000 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., Lansing, MI) 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 seconds. 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 minutes until thoroughly blended. Fifty microliters of slurry was spread over 10 cm x 10 cm clean stainless steel and plastic surfaces and allowed to dry for one hour. 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*

Probability of Detection (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 limit of detection (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 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 section. 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. 100% positive results were observed at 2.5 µg/100cm² 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.

_Determination of Deoxynivalenol in DDGs by Lateral Flow and HPLC_
Since DDGs are a byproduct of ethanol production from corn, they can be contaminated by heat stable mycotoxins if the corn used was infected by toxigenic fungus. Often DDGs are used as feed additives, 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 standard deviation 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 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 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. Standard deviations for the LFD results were < 0.8 ppm, except for one sample where the SD was 1.2 ppm. In general, standard deviations for the mean HPLC results were greater than LFD but HPLC test results includes interlaboratory variability.

| Sample | Mean LFD (ppm) | LFD S.D. |
|--------|----------------|----------|
| DON-3  | <0.3           | NM       |
| DON-5  | 11.8           | 0.8      |
| DON-9  | 6.4            | 0.2      |
| DON-1  | 12.3           | 1.2      |
| DON-2  | 10.7           | 0.6      |
| DON-12 | 12.7           | 0.6      |
A bar chart depiction of results is also shown in Figure 2. The error bars in Figure 2 are ± one standard deviation. Except for sample DON-13 and DON-14, all the LFD results were within one standard deviation 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 standard deviations for replicate measurements.

**General Sanitation Monitoring**

(a) Direct Measurement of Adenosine Triphosphate Standards

The general sanitation assay described in the Methods section was used which detects ATP through 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. 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 standard deviations for those trials is 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 S.D. for 0, 12.5, 25.0 and 100.0 femtomoles 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 (R²) of 0.99 and a slope of 7.5 RLU/femtomole of ATP. The LOD determined by the equation as described in Methods was 6.2 femtomoles of ATP.

(b) 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 S.D. from three samplers with 300 femtomoles of ATP pipetted directly onto the swabs of the samplers. The table provides assay results for the chemiluminescent assay described in the Methods Section and four other types of ATP sanitation samplers that use woven polymer swabs for sampling. Row 2 lists the mean RLU and S.D. from 10 samplers where the same mass of ATP was deposited over a previously cleaned 10 cm x 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 for using the flat sampling pads. Coefficients of variation determined from
the RLU and standard deviation 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.

(c) Recovery of Dried Orange Juice from a 4”x4” 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 cm x 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 cm x 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 Methods 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 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 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 2 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 were 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.

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Tables

Table 1: Probability of Detection (POD) with 95% Upper and Lower Confidence Levels (UCL/LCL) of Gluten Residues in Clean in Place (CIP) Rinse Using Reveal® Gluten 3D Lateral Flow Assay.

| Concentration (ppm Gluten) | n  | Positive Results | POD | LCL | UCL |
|---------------------------|----|------------------|-----|-----|-----|
| 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%|

Table 2: Recovery of Gluten Residues in Working Strength CIP Cleaner.

| Concentration (ppm Gluten) | % Positive Results | Average Line Intensity |
|---------------------------|--------------------|------------------------|
| 0                         | 0%                 | 0                      |
| 2.5                       | 100%               | 1                      |
| 5                         | 100%               | 2.1                    |
| 10                        | 100%               | 3.1                    |

Table 3: Recovery of Gluten Residues on Wet and Dry Surfaces Using Surface Swab Recovery Method.

| Concentration (ppm Gluten) | Wet Surface | Dry Surface |
|---------------------------|-------------|-------------|
|                           | % Positive Results | Average Test Line Intensity | % Positive Results | Average Test Line Intensity |

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Table 4: Recovery of Gluten from Spiked Rice Flour Using Food Extraction Method for the Reveal® 3-D Gluten Lateral Flow Assay.

| Concentration (ppm Gluten) | n  | % Positive Results |
|----------------------------|----|-------------------|
| 0                          | 25 | 0%                |
| 2.5                        | 5  | 0%                |
| 5                          | 25 | 100%              |
| 10                         | 5  | 100%              |
| 15                         | 5  | 100%              |

Table 5: Selectivity Data Demonstrating Results of an Un-spiked Commodity in Comparison to the Same Commodity Spiked at 10ppm 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 6. Summary of Mean Deoxynivalenol Results Obtained by Lateral Flow Devices (LFD) and HPLC for Several DDGs Samples

| Sample  | Mean LFD (ppm) | LFD S.D. | Mean HPLC (ppm) | HPLC S.D. | % Difference LFD vs HPLC |
|---------|----------------|----------|-----------------|-----------|--------------------------|
| DON-3   | <0.3           | NM       | 0.3             | 0.2       | NM                       |
| 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%                       |
| Sample # | RLU at each [ATP] femtomoles | 0  | 12.5 | 25  | 100 |
|----------|-------------------------------|----|------|-----|-----|
| 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 |

Table 7. Surface Sanitation Sampler Response for ATP Standards Pipetted Directly onto Sampler Swabs
Table 8. Mean Response for Recovery of 300 femtomoles of ATP Deposited and Dried on a Surface Relative to Direct Addition to the Sampler

|                  | Flat Sampling Pad | Swab Brand 2 | Swab Brand 3 | Swab Brand 4 | Swab Brand 5 |
|------------------|-------------------|--------------|--------------|--------------|--------------|
| Mean RLU Direct Addition to Sampler | 1038.0 ± 86.1 | 1461.0 ± 63.0 | 624.3 ± 65.3 | 5900.3 ± 7590.68 | 1472.3 ± 432.3 |
| Mean RLU Recovered from Surface | 339.5 ± 59.3 | 21.5 ± 49.8 ± 21.5 | 11.0 | 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”x4” Stainless Steel Surfaces

| Recovery of Orange Juice from 4”x4” Stainless Steel (Mean RLU, S.D. and C.V. %) |
| Orange Juice | Assay Described in Methods | Brand 2 | Brand 3 | Brand 4 | Brand 5 |
|--------------|-----------------------------|---------|---------|---------|---------|
| 1:20         | 46728.9                      | 10294.9 | 3879.1  | 218089.2 |
|              | 7065.5                       | 6160.1  | 1845.6  | 53281.4 |
|              | 15.1%                        | 59.8%   | 47.6%   | 24.4%   |
| 1:100        | 9995.1                       | 2544.2  | 593.2   | 101990.7 |
|              | 2123.8                       | 1112.0  | 359.2   | 58153.6 |
|              | 21.2%                        | 43.7%   | 60.6%   | 57.0%   |
|              | 60.6%                        |         |         |         |
| 1:1000       | 874.3                        | 447.1   | 147.4   | 54438.2 |
|              | 137.9                        | 195.5   | 55.1    | 19605.1 |
|              | 15.8%                        | 43.7%   | 37.4%   | 36.0%   |
|              | 85.3%                        |         |         |         |
| 1:10000      | 92.3                         | 122.2   | 11.6    | 11118.3 |
|              | 22.8                         | 64.6    | 5.9     | 4400.8  |
|              | 24.7%                        | 52.8%   | 51.0%   | 39.6%   |
|              | 74.6%                        |         |         |         |

Table 10. Detection of Different Masses of Bovine Serum Albumin by Colorimetric Assay

| 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   |
Table 11. Overall Results for Colorimetric Assay Detection of BSA Directly Added to Samplers by 3 Operators on 2 Days with 2 Lots of Samplers

| Mass BSA | Lot 1 | Lot 2 | Overall POD |
|----------|-------|-------|-------------|
| 0 μg     | 10.0% | 3.3%  | 6.7%        |
| 5 μg     | 100.0%| 100.0%| 100.0%      |
| 10 μg    | 100.0%| 100.0%| 100.0%      |
| 20 μg    | 100.0%| 100.0%| 100.0%      |
| 50 μg    | 96.7% | 100.0%| 98.3%       |
| 100 μg   | 100.0%| 100.0%| 100.0%      |
| 200 μg   | 100.0%| 100.0%| 100.0%      |

Table 12. Overall Results for Colorimetric Assay Detection of BSA from Stainless Steel Surfaces by 3 Operators on 2 Days with 2 Lots of Samplers

| BSA Recovery from Surface |
|---------------------------|
| 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 | 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
# Food Recovery from Surface

|     | Plastic |     | Steel |     |     |
|-----|---------|-----|-------|-----|-----|
|     | Neg     | Yogurt | Beef  | 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 | 0.0% | 100.0% | 88.3% | 0.0% | 98.3% | 98.3% |
Figure 1: Probability of Detection (POD) with 95% Upper and Lower Confidence Levels (UCL/LCL) of Gluten Residues in Clean in Place (CIP) Rinse Using Reveal Gluten 3D Lateral Flow Assay.

149x136mm (96 x 96 DPI)
Figure 2. Concentration (ppm) of Deoxynivalenol (DON) in Dry Distillers Grain Solubles (DDGs) by LFD and HPLC.

188x136mm (96 x 96 DPI)
Figure 3. Assay Response (RLU) Versus [ATP] femtomoles

149x136mm (96 x 96 DPI)