

Evaluation of Commercial Milk-Specific Lateral Flow Devices

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ABSTRACT

The requirement for validation of allergen cleaning processes is increasing. The use of lateral flow devices (LFDs) to identify allergens has rapidly expanded, but the best practices for use of these devices are still developing. The goal of this study was to compare commercially available milk-specific LFDs and a general protein identification method. Five milk proteins and seven milk-derived ingredients were tested at several concentrations with eight milk-specific LFDs and a general protein identification kit. Nonfat dry milk (NFDM) was prepared at 100 to 10,000 ppm of milk protein and analyzed by the LFDs to determine the concentration at which a false-negative result (overload concentration or hook effect) was obtained. NFDM was also prepared in 0.025 M phosphate-buffered saline (pH 7.4, 0.85% NaCl) and applied to stainless steel panels (100, 30, 10, or 3 μ g of NFDM protein) with various drying methods and sampled with various swab methods to determine the level of detectability. Several total milk LFD kits did not detect whey proteins or whey-derived ingredients. The overload concentration of the various kits ranged from 100 to 10,000 ppm of milk protein. The small dynamic range observed for some kits would necessitate multiple dilutions of a sample to ensure that the result would fall within the range of detection. For swab sampling of stainless steel for LFD analysis, milk protein residues from surfaces onto which the residues were dried with high heat were more difficult to detect than were residues dried with low heat. No differences in sensitivity were observed as a result of moistening the residue or the swab before sampling. These results highlight the importance of understanding the detection capabilities of LFDs as indicated by the variability in the performance of the milk-specific LFDs tested.

Key words: Allergy; Detection; Lateral flow device; Milk; Rapid method

Food allergies are a growing concern for many individuals. Milk allergy, which is one of the most common food allergies, affects 2.5% of infants and children and 0.3% of adults in North America (21). Milk is a common allergenic food, and various milk-derived ingredients are among the most commonly used ingredients in the food industry (5, 22). The only way to prevent an allergic reaction is to avoid the offending allergenic food. However, the implementation of a safe and effective avoidance diet is difficult for milk-allergic individuals because milk is the most common undeclared allergen, has led to allergic reactions due to cross-contact, and is found in products with and without advisory labeling and in processed foods with difficult equipment cleaning requirements such as chocolate (2, 6, 7). Undeclared milk allergens can be in the form of milk in raw ingredients, processing aids, reworked product, or carryover from shared equipment (22).

To protect the milk-allergic consumer, food manufacturers using milk or milk-derived ingredients should develop allergen control plans. The validation of the effectiveness of those plans involves the use of methods to detect milk protein residues, which include the testing of equipment, food processing surfaces, rinse water, ingredients, or

finished product. The U.S. Food and Drug Administration Food Safety Modernization Act (23) requires the development of effective allergen control plans and testing procedures, which when properly implemented can aid in plan validation.

Rapid and specific methods such as lateral flow devices (LFDs) are being more commonly used in the food industry to monitor cleaning of food processing equipment and food product contamination (1, 15–17). LFDs are immunochromatographic allergen detection devices that detect specific antibody-antigen reactions. With LFDs, the first step involves extraction of the analyte from the sample, which can be a swab, rinse water, or a food or food ingredient. With commercial milk LFDs, kit instructions range from hand shaking at room temperature to vortexing and boiling the sample. The extracted sample is introduced onto the device, and antigens (allergens) in the sample bind to conjugated antibody (immunoglobulin [Ig] G conjugated to gold or colored latex particles that aid in the development of a colored test line) in the test zone of the strip. The antigen-conjugated antibody complex then travels through the test zone and binds to an area of the strip where allergen-specific IgG is fixed, and a positive test line is formed. Any extra conjugated antibody continues through the LFD and is bound at the control line by species-specific IgG (1).

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The rise in use of commercial LFD kits came rapidly (19). The first allergen rapid immunoassay was developed for peanuts in 1997 (11). In 2004, two peanut LFDs were commercially released and joined a handful of gluten LFDs on the market (25). Currently, at least eight companies produce LFDs targeting milk, casein, or β -lactoglobulin (BLG; a whey protein), and LFDs are available for many other allergens. Many factors can affect test results such as manufacturing issues, operator errors, effects of the food matrix, environmental factors, sample manipulation, and cross-reactivity (26). An additional limitation of LFDs is the high dose hook effect or overloading of the device, which occurs when the allergen concentration in the sample is very high and false-negative results are obtained (1, 13). Matrix interference influences the detection of peanut in chocolate samples tested with LFDs as compared with sandwich enzyme-linked immunosorbent assays (ELISAs), whereas cookies had similar numbers of positive results with ELISAs and LFDs (14). Similarly, an r-Biopharm milk LFD was validated with milk, ice cream, and milk powder applied and dried on a surface (16); the sensitivity of the test for milk powder was lower than that for the other products. In an interlaboratory validation study of two peanut LFDs, the sensitivity of the kits approached that of sandwich ELISA kits (24). However, differences in the frequency of false-positive results were found between the two commercial methods, which may be attributable to differences in antibody type and quality (24). These issues are important to take into account when selecting a suitable LFD kit. The nature of the sample for LFD testing also should be considered. Some kit inserts specify adjustments to the protocol for sorbets and thick or viscous products, which may interfere with sample movement throughout the LFD by capillary action. Samples derived from swabbing can produce variable results because swabbing is a subjective procedure. Development of consistent swabbing procedures may reduce variability between test operators (27).

Although LFDs are suitable for qualitative screening in the food processing environment, allergen test kits and methods must be validated both in interlaboratory studies and by the end user with actual products containing the allergenic ingredient of interest (12, 15).

The objective of this study was to investigate the milk protein and milk-derived ingredient specificity, overload level, and swab sample processing characteristics of commercial milk-specific LFDs. This information will allow comparisons between the different kits that will enable the analysts in the food industry to select an appropriate LFD kit for detection and interpretation of results.

MATERIALS AND METHODS

Purified milk proteins. Purified protein fractions of the bovine milk proteins α -, β -, and κ -casein, BLG, and α -lactalbumin (Sigma-Aldrich, St. Louis, MO) at ≥ 70 , ≥ 98 , ≥ 70 , ≥ 90 , and $\geq 85\%$ purity, respectively, were used to prepare 100 ppm of protein (wt/vol) solutions in 0.025 M phosphate-buffered saline (PBS; pH 7.4, 0.85% saline). The solutions were prepared assuming 100% purity of protein sources. The 100-ppm protein solution was further diluted to prepare protein solutions with 1 and 10 ppm (vol/vol). The 0.025 M PBS solution was used as a

negative control. The 1, 10, and 100 ppm of protein samples were run in triplicate with each LFD described below except in the analysis with the Neogen Reveal Total Milk kit, in which the samples were extracted in duplicate and then each extract was tested in duplicate wells.

Milk-derived ingredients. Seven milk-derived ingredients were analyzed: low heat-processed nonfat dry milk (NFDM; Darigold, Seattle, WA), whey protein concentrate with 34 and 80% protein (WPC34 and WPC80; Erie Foods International, Erie, IL), sodium caseinate (Erie Foods), sweet whey powder (Grande Custom Ingredients Group, Lomira, WI), acid whey powder (Saputo Dairy Foods, Dallas, TX), and milk protein concentrate with 80% protein (MPC80; Idaho Milk Products, Jerome, ID). The protein concentration of each ingredient was determined through analysis on an FP-528 protein/nitrogen determinator (LECO Corp., St. Joseph, MI) according to the manufacturer's instructions.

Each milk-derived ingredient was used to prepare a 100-ppm protein (wt/vol) solution in 0.025 M PBS. Each solution was stirred for 15 min and then placed for 30 min in a 60°C shaking water bath. The solutions were diluted in PBS to prepare 75, 25, 10, 5, and 1 ppm of protein (vol/vol) samples. Each sample was analyzed in triplicate as a liquid sample as described in the LFD kit instructions. The general protein kit was used by directly pipetting 0.15 ml of the sample onto the swab head. See Supplemental Tables 1 through 9 for complete results (all supplemental material is available at [http://farrp.unl.edu/publication supplement](http://farrp.unl.edu/publication%20supplement)).

Analysis of overload concentration hook effect. Samples of NFDM were prepared as previously described in 0.025 M PBS at 100 to 10,000 ppm of protein and tested in triplicate with each LFD as a liquid sample. The general protein kit was used by directly pipetting 0.15 ml of the sample onto the swab head. The overload concentration was determined by the absence or depression of the test line and/or the absence or depression of the overload line when applicable.

Analysis of swab sampling conditions. A 1,000-ppm NFDM protein solution was prepared in 0.025 M PBS and directly diluted with the same PBS solution to prepare 300, 100, and 30 ppm of NFDM protein solutions. A 0.1-ml aliquot of each sample was applied to a panel (3 by 5 in. [7.6 by 12.7 cm]) made of 316 grade stainless steel (Ecolab, St. Paul, MN) and spread with a pipet tip to cover an area of 7 by 7 cm. All samples were collected with swabs using a crosshatch technique over the entire soiled area. Triplicate soil panels were prepared for each protein concentration, and the soiled panels were treated in one of five ways.

(i) For method A, the NFDM soil was gently dried at 37°C for approximately 13 min. The dry soil was moistened with 0.1 ml of purified water processed through a reverse osmosis and deionizer system (ROD water), and a sample from the soiled area was then collected with a dry swab provided with the kit.

(ii) For method B, the NFDM soil was gently dried at 37°C for approximately 13 min. The swab head was directly moistened with 0.1 ml of moistener or ROD water as per the kit instructions and then used to collect the dry soil sample.

(iii) For method C, the NFDM soil was dried at high temperature in a 350°F (177°C) oven for 10 min. After cooling, 0.1 ml of ROD water was applied to the surface, and the sample was collected with a dry swab.

(iv) For method D, the NFDM soil was dried at high temperature in a 350°F oven for 10 min. After cooling, 0.1 ml of

ROD water or kit-provided moistener was applied to the swab head, which was then used to collect the dry soil sample.

(v) For method E, the NFDM soil samples were collected immediately (while still moist) with a dry swab.

Initially, each kit was tested with the swab provided with the kit. The Morinaga kit does not provide swabs, so a cotton-tipped PurSwab 867-WC (Puritan Medical Products Company, Guilford, ME) was used. The entire experiment was repeated as described above using the Neogen Allergen Environmental Swab (NAES; Neogen Corp., Lansing, MI) with each kit (see Supplemental Table 10 for results). The 3M Clean-Trace Surface Protein (Allergen) kit (3M Health Care, St. Paul, MN) was excluded from the analysis using the NAES because the 3M kit comes with a specific swab for its container.

The stainless steel panels were cleaned between experiments with a warm solution of Micro-90 (International Products Corp., Burlington, NJ), a concentrated alkaline cleaning solution commonly used in the laboratory. This cleaning procedure was validated with the Neogen Veratox Total Milk Allergen kit.

LFD and general protein kits. Eight commercially available total milk, casein, or BLG LFD kits and one total protein kit were used for analysis: Romer AgraStrip Total Milk, Romer AgraStrip Casein, and Romer AgraStrip BLG (Romer Labs, Runcorn, Cheshire, UK), Neogen Reveal 3-D Total Milk and Neogen Reveal Total Milk (Neogen), Bioavid Diagnostics Lateral Flow Milk (Bioavid Diagnostics, Wendelsheim, Germany), Elution Technologies Bovine Total Milk (Elution Technologies, Colchester, VT), Morinaga Casein (Morinaga Institute of Biological Science, Yokohama, Japan), and 3M Clean-Trace Surface Protein Allergen (3M Health Care). The kit specifications provided by each manufacturer are provided in Table 1.

The instructions for each LFD were followed with the following modifications. The Neogen Reveal 3-D extraction buffer packets were pooled, and 3.75 ml of extraction buffer was placed into each sample vial. The Elution Technologies instructions described wetting the swab head for swab sampling; this step was standardized by applying 0.1 ml of extraction buffer from the tube to the swab head, and the instruction to swirl several times was standardized to vortexing for 10 s. The NAESs were returned to their swab containers with 5 ml of preheated working solution and then processed by placing all swab containers in a sealed plastic bag into a 60°C shaking water bath for 15 min. After cooling to room temperature, a 1-ml aliquot of the extracted solution was centrifuged for 10 min at 13,000 rpm (Sorvall Legend Micro 17 Centrifuge, ThermoScientific, Waltham, MA).

The qualitative results of each kit were read visually at the specified time (3 to 15 min depending on the kit) and interpreted according to each instruction manual. Triplicate samples were tested at each concentration. The visual observation of two or more test lines within a triplicate sample was considered a positive result. The visual observation of one or no test line within a triplicate sample was considered a negative result. The Elution and Neogen Reveal 3-D kits have an overload line, but the other LFD kits do not.

RESULTS AND DISCUSSION

Several total milk LFDs did not detect whey proteins. The protein specificity of the various LFDs was analyzed. The Romer, Neogen, Bioavid, and Elution total milk kits did not detect proteins from the whey fraction of milk, α -lactalbumin and BLG (Table 2). The discrepancy

between the advertised detection capabilities (Table 1) and the actual results is a concern for the customer using a total milk kit to detect whey ingredients. The casein and BLG kits successfully detected only their target proteins. No kit was able to detect α -lactalbumin. This issue is a concern because α -lactalbumin is being used more often in production and ingredient development and may be the sole milk-related target of interest in certain products (3, 10).

LFD sensitivity to milk-derived ingredients was variable. The sensitivity of the LFDs to NFDM ranged from 1 ppm of protein (Romer Casein and Bioavid) to 5 ppm of protein (Romer BLG, Romer Total Milk, Neogen Reveal, Neogen Reveal 3-D, Elution, and Morinaga) (Table 3). The lowest concentration of NFDM that could be detected with the 3M kit (a general protein method) was 75 ppm of protein. Although general protein swabs have lower sensitivity, they were an effective method of detection when compared with visual inspection, ELISAs, and ATP tests in a study of allergen detection after dry cleaning methods (8).

LFD specificity for milk-derived ingredients was variable. Six concentrations of each ingredient were analyzed; 75 ppm of protein was determined to be representative of the overall results because it was above the sensitivity of the various LFDs and below the beginning of the hook effect (Table 4). The test line intensity differed by kit and sometimes throughout the concentration range tested. Some kits, particularly Romer BLG, Neogen Reveal 3-D, and Bioavid, had test lines that increased and then decreased in intensity as the protein concentration of the sample increased. This observation may have been the beginning of the hook effect in which there is no longer an excess of antibodies because of high concentrations of analyte, which inverts the dose-response curve (1, 13). LFDs are generally used to verify equipment cleanliness, so the user must understand that a faint positive test line does not necessarily mean a low level of contamination and that further cleaning should be initiated whenever any type of positive result is found (9, 22).

Similar to the milk protein specificity results, certain LFDs did not detect milk-derived ingredients made of whey. The Neogen Reveal, Neogen Reveal 3-D, and Elution kits did not detect WPC34, WPC80, or sweet whey at 75 ppm of protein. Soon after these results were obtained, the Elution kit was redesigned; in subsequent retesting of the whey ingredients, the kit was able to detect very faint positive results for 75 ppm of protein. Acid whey was faintly detected by these kits. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of the milk-derived ingredients revealed that the acid whey sample was relatively not as pure and displayed trace protein bands at the molecular weights of α -, β -, and κ -casein (data not shown). The Romer Casein and Morinaga kits were able to positively detect the acid whey sample.

To select the LFD kit that is the best fit for any specific purpose, kit users should determine which kits actually detect milk residues in their products, ingredients, or environmental

TABLE 1. Lateral flow device kit specifications provided by the manufacturers

LFD kit	Kit specifications			
	LOD	LOD (μg) ^a	Range of detection	Reported overload concn
Romer AgraStrip Total Milk	1 ppm of milk protein	0.025	1–10,000 ppm of milk protein; 3–30,000 ppm of skimmed milk powder	>10,000 ppm (250 μg of milk protein)
Romer AgraStrip Casein	1 ppm of casein	0.025	1–10,000 ppm of casein	>10,000 ppm
Romer AgraStrip β -lactoglobulin	0.5 ppm of BLG	0.0125	0.5–100 ppm of BLG; 15–3,000 ppm of skimmed milk powder	>10,000 ppm
Neogen Reveal Total Milk	5 ppm of milk residue	0.05	NA ^b	NA
Neogen Reveal 3-D Total Milk	5–10 ppm of total milk	0.146–0.292	5–>1,000 ppm	>1,000 ppm
Bioavid Diagnostics Milk	1 ppm of milk protein	0.5	NA	>1,000 ppm of milk
Elution Technologies Bovine Total Milk	1–2 ppm of total milk	0.01–0.02 ^c	<1,000 ppm of NIST 1846	>50% bovine milk
Morinaga Casein	5 ppm of milk protein	0.005	NA	Very high concentrations of casein
3M Clean-Trace Surface Protein (Allergen) ^d	3 μg of total protein or 10–20 μg of whole milk powder	2.59–5.18 μg of whole milk protein ^e	NA	NA

^a LOD, limit of detection. The LOD was converted from ppm to μg from the dilution and sample preparation information provided in the kit instruction manuals.

^b NA, not applicable (information not provided in the kit specifications).

^c Calculated with Elution Technologies adjusted protocol.

^d A general protein kit, not a lateral flow device.

^e LOD calculated from protein concentration information included in the instruction manual of reference material NIST SRM8435.

TABLE 2. *Milk protein specificity*

LFD kit	Milk protein specificity ^a				
	α -Casein	β -Casein	κ -Casein	β -Lactoglobulin	α -Lactalbumin
Romer AgraStrip Total Milk	Yes	Yes	Yes	±	No
Romer AgraStrip Casein	Yes	Yes	Yes	No	No
Romer AgraStrip BLG	No	No	No	±	No
Neogen Reveal Total Milk	Yes	Yes	Yes ^b	No	No
Neogen Reveal 3-D Total Milk	Yes	Yes	Yes ^b	No	No
Bioavid Diagnostics Milk	Yes	Yes	±	No	No
Elution Technologies Bovine Total Milk	±	No	±	No	No
Morinaga Casein	Yes ^b	Yes	Yes	No	No

^a Protein solutions were tested at 100, 10, and 1 ppm of protein. Results listed here indicate whether the kit was able to detect at least two positive results at one or more of the concentrations. Each solution was tested in triplicate. ±, a very faint test line that may be interpreted differently by different users.

^b Intrakit sensitivity: kit was able to positively detect (at least two replicates) this protein at a lower concentration than those used for the other proteins tested.

samples and should understand the dynamic range of the LFD and the effects of overload on the LFD results.

Commercial LFDs had variable overload concentrations. Very high concentrations of allergen can overload an LFD and lead to a false-negative result (1, 13). This hook effect is observed in LFDs when the test line decreases in intensity and eventually does not form as the analyte (allergen) load of the sample increases. The concentration of milk protein that would cause an overload condition might differ among LFD kits. The LFDs that were evaluated had overload concentrations of 100 and 10,000 (the highest concentration tested) ppm of NFDM protein (Table 5). Kits that have very narrow ranges of detection would require the user to test several product dilutions to ensure that one of the dilutions would fall within the range of detection. All test kits analyzed except Neogen Reveal 3-D come with extra extraction solution that could be used to dilute the sample before testing. These dilutions would also be necessary when

sampling dirty equipment (positive control) to ensure that the product or ingredient of interest is detectable by the LFD kit.

To combat the hook effect, two companies produce LFDs (Neogen Reveal 3-D and Elution) with an additional overload line. Both of these kits became overloaded at about the same protein concentration (Table 5). When the test line and the overload line do not develop, the sample probably had a high allergen load and further dilutions of the product should be tested. With the Neogen Reveal 3-D kit, the overload line decreased to intensities less than the test line in samples with higher concentrations of protein, which would lead the user to believe that the sample was at the upper detectable limit, >1,000 ppm. This result was obtained for both of the samples that were positive and then negative (the overload line decreased in intensity and the test line was never positive). With the Elution kit, the overload line decreased in intensity with the higher concentrations of MPC80 and NFDM tested.

The Morinaga and 3M kits were not subject to the hook effect over the concentration range tested. The 3M kit

TABLE 3. *Milk-derived ingredient sensitivity*

LFD kit	Kit sensitivity (ppm of protein, result) ^a						
	NFDM	MPC80	Sodium caseinate	WPC34	WPC80	Sweet whey	Acid whey
Romer AgraStrip Total Milk	5, Pos	5, Pos	5, Pos	5, Pos	5, Pos	5, Pos	5, Pos
Romer AgraStrip Casein	1, F pos	1, F pos	5, Pos	5, Pos	1, F pos	5, Pos	5, F pos
Romer AgraStrip BLG	5, Pos	5, Pos	Neg	5, F pos	5, F pos	5, Pos	5, Pos
Neogen Reveal Total Milk	5, ±	5, ±	5, ±	Neg	Neg	Neg	25, ±
Neogen Reveal 3-D Total Milk	5, Pos	5, F pos	5, F pos	Neg	Neg	Neg	25, ±
Bioavid Diagnostics Milk	1, F pos	1, ±	1, Pos	1, F pos	1, F pos	5, Pos	1, F pos
Elution Technologies Bovine Total Milk ^b	5, ±	5, ±	10, ±	Neg	Neg	Neg	25, ±
Morinaga Casein	5, F pos	5, F pos	5, F pos	75, ±	100, ±	Neg	25, ±
3M Clean-Trace Surface Protein (Allergen)	75, Pos	75, pos	75, pos	25, ±	25, ±	25, ±	25, Pos

^a Sensitivity was determined as the lowest concentration at which two or more replicates produced positive results. F pos, faint positive; ±, a very faint test line that may be interpreted differently by different users.

^b Elution Technologies Bovine Total Milk kit was redesigned during the study period; thus, some samples were retested with the new kit. No change in detection was determined for NFDM, but WPC34, WPC80, and sweet whey were initially given a ± result at 75 ppm of protein with the new kit.

TABLE 4. Milk-derived ingredient specificity at 75 ppm of protein

LFD kit	Result (no. positive/no. tested) ^a						
	NFDM	MPC80	Sodium caseinate	WPC34	WPC80	Sweet whey	Acid whey
Romer AgraStrip Total Milk	Pos (3/3)	Pos (3/3)	Pos (3/3)	Pos (3/3)	Pos (3/3)	Pos (3/3)	Pos (3/3)
Romer AgraStrip Casein	Pos (3/3)	Pos (3/3)	Pos (3/3)	F pos (3/3)	Pos (3/3)	Pos (3/3)	Pos (3/3)
Romer AgraStrip BLG	Pos (3/3)	Pos (3/3)	Neg (0/3)	Neg (0/3)	F pos (2/3)	F pos (3/3)	± (3/3)
Neogen Reveal Total Milk	Pos (3/3)	Pos (3/3)	Pos (3/3)	Neg (0/3)	Neg (0/3)	Neg (1/3)	± (3/3)
Neogen Reveal 3-D Total Milk	Pos (3/3), O < T ^b	Pos (3/3), O << T	Pos (3/3), O < T	Neg (0/3), O ±	Neg (0/3), O ±	Neg (0/3)	F pos (3/3)
Bioavid Diagnostics Milk	F pos (3/3)	F pos (3/3)	Pos (3/3)	F pos (3/3)	F pos (3/3)	± (2/3)	F pos (2/3)
Elution Technologies Bovine Total Milk ^c	F pos (3/3)	F pos (3/3)	± (2/3)	Neg (0/3)	Neg (0/3)	Neg (0/3)	± (2/3)
Morinaga Casein	Pos (3/3)	Pos (3/3)	Pos (3/3)	± (2/3)	Neg (1/3)	Neg (0/3)	Pos (3/3)
3M Clean-Trace Surface Protein (Allergen)	Pos (3/3)	Pos (3/3)	Pos (3/3)	Pos (3/3)	Pos (3/3)	Pos (3/3)	Pos (3/3)

^a Protein concentration of each ingredient was determined through LECO Dumas protein analysis. Solutions were tested at 100, 75, 25, 10, 5, and 1 ppm of protein. Analysis was performed in triplicate, and the result listed was recorded for two or more of the replicates. F pos, faint positive; ±, a very faint test line that may be interpreted differently by different users.

^b O, overload line; T, test line. Neogen stated that when O intensity is less than T intensity, the LFD is overloaded.

^c Elution Technologies Bovine Total Milk kit was redesigned during the study period; thus, some samples were retested with the new kit. WPC34, WPC80, and sweet whey were given a ± result at 75 ppm of protein.

produced strongly positive results throughout the range tested because the analysis relies on the Biuret method in which higher protein concentrations yield more intense color development. The consistent test line intensity observed with the Morinaga kit is most likely a result of proprietary differences in kit development.

TABLE 5. Overload concentrations of LFDs with NFDM

LFD kit	Overload concn (ppm of NFDM protein) ^a	Range of positive detection before overload (ppm of NFDM protein)
Romer AgraStrip Total Milk	2,500	5–1,000
Romer AgraStrip Casein	~10,000 ^b	1–10,000
Romer AgraStrip BLG	1,000	5–500
Neogen Reveal Total Milk	~10,000 ^b	5–10,000
Neogen Reveal 3-D Total Milk	1,000	5–750
Bioavid Diagnostics Milk	100	1–75
Elution Technologies Bovine Total Milk	750	5–500
Morinaga Casein	>10,000 ^b	5–10,000
3M Clean-Trace Surface Protein (Allergen)	>10,000 ^b	75–10,000

^a Overload concentration was defined as the lowest concentration at which the absence or depression of the test line and/or the absence or depression of the overload line, when applicable, occurred.

^b Overload was not observed at 10,000 ppm of NFDM protein.

Influence of swab sampling conditions on LFD detection. Experiments were conducted in which the NAES was used with all of the milk LFD kits to remove any differences that might be attributed to the swab when collecting samples from a stainless steel surface (Supplemental Table 10). In general, the swabs provided with the kits resulted in slightly higher sensitivity for NFDM detection than did the NAES (Table 6 and Supplemental Table 10). With all of the soil preparation methods used in this experiment, soils were easily removed from the surface. The NAES was better at scraping than at absorbing soils. When soil was crusty or extensively baked onto the food processing surface, the NAES may be a suitable swab for scraping the soil from the surface. When the soil is a liquid or still moist, a more absorbent swab would be a better choice.

The wet soil sampled with the dry swab (method E) had the highest sensitivity, followed by the low temperature oven-dried samples and then the high temperature oven-dried samples. Oven drying may have had thermal effects on the protein, which can lower detection by ELISA (4). The baked-on soil may also have been more difficult to remove from the panel with the swab and/or may be less soluble in the extraction solution (20). Swabbing practices generally recommend moistening a swab to saturation before swabbing, but many LFD kits have variable instructions about moistening the surface or moistening the swab head (27). In general, no differences in sensitivity were observed between moistening the swab and moistening the surface when swabbing dry soils. Swabbing the same area with two swabs was not investigated in this study but has previously been found to increase recovery (12, 18, 27). A consistent swabbing method should be developed for an allergen cleaning program (9).

TABLE 6. Effect of swabbing conditions on sensitivity of LFD kits tested with kit-provided swab^a

LFD kit	Sensitivity (μg of NFDM protein, result) ^b				
	Method A	Method B	Method C	Method D	Method E
Romer AgraStrip Total Milk	3, $\pm$ Delay	3, $\pm$	10, Pos	10, F pos	3, F pos
Romer AgraStrip Casein	3, F pos	3, F pos	3, Delayed F pos	3, Delayed F pos	3, Pos
Romer AgraStrip BLG	3, $\pm$	10, F pos	10, $\pm$	10, $\pm$	3, F pos
Neogen Reveal Total Milk	30, Pos	10, $\pm$	30, $\pm$	30, $\pm$	30, $\pm$
Neogen Reveal 3-D Total Milk	10, F pos	10, F pos	10, F pos	10, $\pm$	3, $\pm$
Bioavid Diagnostics Milk	3, F pos	3, F pos	3, Pos	3, F pos	3, Pos
Elution Technologies Bovine Total Milk	3, F pos	3, $\pm$	3, $\pm$	3, $\pm$	3, F pos
Morinaga Casein	3, F pos	3, F pos	3, F pos	3, F pos	3, F pos
3M Clean-Trace Surface Protein (Allergen)	10, $\pm$	10, F pos	10, F pos	10, $\pm$	10, F pos

^a This experiment was replicated using the Neogen Allergen Environmental Swab (Supplemental Table 10) with each kit, except the 3M Clean-Trace Surface Protein (Allergen). The sensitivity was similar or lower than that obtained when samples were collected with the swabs provided with the kit.

^b Overall result reported for sensitivity was the lowest concentration positively detected two or more times when samples were analyzed in triplicate. For method A, the NFDM soil was gently dried at 37°C for approximately 13 min, the dry soil was moistened with 0.1 ml of ROD water, and the soiled area was sampled with a dry swab provided with the kit. For method B, the NFDM soil was gently dried at 37°C for approximately 13 min, the swab head was directly moistened with 0.1 ml of moistener or ROD water as directed in the kit instructions, and the dry soil was then swabbed. For method C, the NFDM soil was dried at high temperature in a 350°F oven for 10 min and then cooled, 0.1 ml of ROD water was applied to the surface, and the soiled area was sampled with a dry swab. For method D, the NFDM soil was dried at high temperature in a 350°F oven for 10 min and then cooled, 0.1 ml of ROD water or kit-provided moistener was applied to the swab head, and the dry soil was then swabbed. For method E, the NFDM soil was sampled immediately (still moist) with a dry swab. All swabbing was conducted using a crosshatch technique.

In conclusion, commercially available milk-specific LFDs differ greatly in their performance. Potential LFD users must understand the detection capabilities and limitations of the various milk LFD kits to make an appropriate selection. Several serious limitations were noted with LFDs. Not all total milk LFDs detected whey proteins or whey-derived ingredients. Some kits had a very narrow range of detection and became overloaded at low protein concentrations. When using swabs to collect samples from production surfaces, the type of soil and type of swab may influence the sensitivity of the LFD. LFDs provide qualitative results, and assumptions about the degree of contamination based on test line intensity should not be made because intensities varied throughout the protein concentration ranges tested.

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REFERENCES

- Baumert, J. L., and D. H. Tran. 2015. Lateral flow devices for detecting allergens in food, p. 219–228. In S. Flanagan (ed.), *Handbook of food allergen detection and control*. Woodhead Publishing, Cambridge.
- Crotty, M. P., and S. L. Taylor. 2010. Risks associated with foods having advisory milk labeling. *J. Allergy Clin. Immunol.* 125:935–937.
- Delavari, B., A. A. Saboury, M. S. Atri, A. Ghasemi, B. Bigdeli, A. Khammari, P. Maghami, A. A. Moosavi-Movahedi, T. Haertlé, and B. Goliaei. 2015. Alpha-lactalbumin: a new carrier for vitamin D₃ food enrichment. *Food Hydrocoll* 45:124–131.
- Downs, M. L., and S. L. Taylor. 2010. Effects of thermal processing on the enzyme-linked immunosorbent assay (ELISA) detection of milk residues in a model food matrix. *J. Agric. Food Chem.* 58:10085–10091.
- Gendel, S. M., N. Khan, and M. Yajnik. 2013. A survey of food allergen control practices in the U.S. food industry. *J. Food Prot.* 76:302–306.
- Gendel, S. M., and J. Zhu. 2013. Analysis of U.S. Food and Drug Administration food allergen recalls after implementation of the food allergen labeling and consumer protection act. *J. Food Prot.* 76:1933–1938.
- Gern, J. E., E. Yang, H. M. Evrard, and H. A. Sampson. 1991. Allergic reactions to milk-contaminated nondairy products. *N. Engl. J. Med.* 324:976–979.
- Jackson, L. S., and F. Al-TaHER. 2010. Efficacy of different dry cleaning methods for removing allergenic foods from food-contact surfaces, P1-47. Abstr. Annu. Meet. IAFP 2010. International Association for Food Protection, Des Moines, IA.
- Jackson, L. S., F. M. Al-TaHER, M. Moorman, J. W. DeVries, R. Tippet, K. M. J. Swanson, T.-J. Fu, R. Salter, G. Dunaif, S. Estes, S. Albillos, and S. M. Gendel. 2008. Cleaning and other control and validation strategies to prevent allergen cross-contact in food-processing operations. *J. Food Prot.* 71:445–458.
- Marella, C., K. Muthukumarappan, and L. E. Metzger. 2011. Evaluation of commercially available, wide-pore ultrafiltration membranes for production of α -lactalbumin-enriched whey protein concentrate. *J. Dairy Sci.* 94:1165–1175.
- Mills, E. N. C., A. Potts, G. W. Plumb, N. Lambert, and M. R. A. Morgan. 1997. Development of a rapid dipstick immunoassay for the detection of peanut contamination of food. *Food Agric. Immunol.* 9:37–50.
- Monaci, L., and A. Visconti. 2010. Immunochemical and DNA-based methods in food allergen analysis and quality assurance perspectives. *Trends Food Sci. Technol.* 21:272–283.
- O'Farrell, B. 2009. Evolution in lateral flow-based immunoassay systems, p. 1–33. In R. C. Wong and H. Y. Tse (ed.), *Lateral flow immunoassay*. Humana Press, New York.

14. Pele, M., M. Brohée, E. Anklaam, and A. J. V. Hengel. 2007. Peanut and hazelnut traces in cookies and chocolates: relationship between analytical results and declaration of food allergens on product labels. *Food Addit. Contam.* 24:1334–1344.
15. Rice, J. A., and A. J. Lupo. 2014. Immunodiagnosics in food allergen testing, p. 13–28. *In* Food allergen testing. John Wiley & Sons, New York.
16. Richter, M., S. Haas-Lauterbach, and K. Schmitt. 2010. Rapid lateral flow tests in a hazard analysis of critical control points–based approach for allergen monitoring. *Qual. Assur. Saf. Crops Foods* 2:165–172.
17. Röder, M., S. Vieths, and T. Holzhauser. 2009. Commercial lateral flow devices for rapid detection of peanut (*Arachis hypogaea*) and hazelnut (*Corylus avellana*) cross-contamination in the industrial production of cookies. *Anal. Bioanal. Chem.* 395:103–109.
18. Schlegel, V., A. Yong, and S. Y. Foo. 2007. Development of a direct sampling method for verifying the cleanliness of equipment shared with peanut products. *Food Control* 18:1494–1500.
19. Schubert-Ullrich, P., J. Rudolf, P. Ansari, B. Galler, M. Führer, A. Molinelli, and S. Baumgartner. 2009. Commercialized rapid immunoanalytical tests for determination of allergenic food proteins: an overview. *Anal. Bioanal. Chem.* 395:69–81.
20. Sharma, G. M., S. E. Khuda, M. Pereira, A. Slate, L. S. Jackson, C. Pardo, K. M. Williams, and T. B. Whitaker. 2013. Development of an incurred combread model for gluten detection by immunoassays. *J. Agric. Food Chem.* 61:12146–12154.
21. Sicherer, S. H., and H. A. Sampson. 2010. Food allergy. *J. Allergy Clin. Immunol.* 125:S116–S125.
22. Taylor, S. L., S. L. Hefle, K. Farnum, S. W. Rizk, J. Yeung, M. E. Barnett, F. Busta, F. R. Shank, R. Newsome, S. Davis, and C. M. Bryant. 2006. Analysis and evaluation of food manufacturing practices used to address allergen concerns. *Compr. Rev. Food Sci. Food Saf.* 5:138–157.
23. U.S. Food and Drug Administration. 2015. Current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food. Docket no. FDA-2011-N-0920. Available at: <https://federalregister.gov/a/2015-21920>. Accessed 18 January 2016.
24. Van Hengel, A. J., and C. Capelletti. 2006. Validation of two commercial lateral flow devices for the detection of peanut proteins in cookies: interlaboratory study. *J. AOAC Int.* 89:462–468.
25. Van Herwijnen, R., and S. Baumgartner. 2006. The use of lateral flow devices to detect food allergens, p. 175–181. *In* S. J. Koppelman and S. L. Hefle (ed.), *Detecting allergens in food*. Woodhead Publishing, Cambridge.
26. Wong, R. C., and H. Y. Tse. 2009. Quantitative, false positive, and false negative issues for lateral flow immunoassays as exemplified by onsite drug screens, p. 185–203. *In* R. C. Wong and H. Y. Tse (ed.), *Lateral flow immunoassay*. Humana Press, New York.
27. Yang, P., K. Burson, D. Feder, and F. Macdonald. 2005. Method development of swab sampling for cleaning validation of a residual active pharmaceutical ingredient. *Pharm. Technol.* 29(1):84–94.

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