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Development of an Immunochromatographic Strip Test for Rapid Detection of Melamine in Raw Milk, Milk Products and Animal Feed

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ABSTRACT: A simple, rapid and sensitive immunogold chromatographic strip test based on a monoclonal antibody was developed for the detection of melamine (MEL) residues in raw milk, milk products and animal feed. The limit of detection was estimated to be 0.05 μg/mL in raw milk, since the detection test line on the strip test completely disappeared at this concentration. The limit of detection was 2 μg/mL (or 2 μg/g) for milk drinks, yogurt, condensed milk, cheese, and animal feed and 1 μg/g for milk powder. Sample pretreatment was simple and rapid, and the results can be obtained within 3−10 min. A parallel analysis of MEL in 52 blind raw milk samples conducted by gas chromatography−mass spectrometry showed comparable results to those obtained from the strip test. The results demonstrate that the developed method is suitable for the onsite determination of MEL residues in a large number of samples.

KEYWORDS: melamine, colloidal gold, strip test, raw milk, milk products, animal feed

INTRODUCTION

Melamine (1,3,5-triazine-2,4,6-triamine, MEL, Figure 1) is a synthetic compound that is widely used as an industrial chemical for the production of plastics, amino resins and flame-retardants.[1−3] Because of its high nitrogen content,[4] the use of MEL as a non-protein nitrogen (NPN) source for cattle was described in a 1958 patent.[5] But during a dietary feeding trial of nitrogen sources for ruminants in 1978, MEL was shown to be difficult for cattle to hydrolyze; therefore, MEL was not an acceptable NPN source or nitrogen supplement for cattle.[6]

The use of MEL can make poor quality protein commodities appear to have high protein content by elevating the total nitrogen level. Recently, MEL was intentionally and illegally added to food or food-related products.[7] MEL as an adulterant can provide a false indication of increased protein concentration during testing procedures, thereby providing a false indication that the material has a high protein content. Although MEL has low oral toxicity,[8] studies showed that high and continuous dietary exposure to MEL can cause renal stones and urinary bladder tumors.[8,9] In 2004, an outbreak of food contaminated with MEL led to kidney failure in cats and dogs.[9] In March 2007, thousands of illnesses and deaths of pets in the United States were demonstrated to be caused by MEL contaminated pet food.[10] In September 2008, infant milk powder adulterated with MEL was found in China; nearly 54000 infants were hospitalized, and at least six infants died.[11] MEL contamination of food or food-related products has become a worldwide concern. In order to guarantee safe food and protect the health of humans, many countries and regions such as the United States, the European Union (EU) and Australia have established a tolerance level for MEL and its analogues in food to be 2.5 mg/kg, with no detectable level of MEL allowed in baby food.[12−14] China has established the tolerance level for MEL to be 1 mg/kg for infant formula and 2.5 mg/kg for milk, milk products and food containing >15% milk.[12−14] Therefore, it is extremely important to monitor MEL in animal feed and animal derived food, and especially in milk and milk products.

Various analytical methods have been developed to analyze for MEL, such as gas chromatography−mass spectrometry (GC−MS),[15−17] high-performance liquid chromatography (HPLC),[18−20] liquid chromatography−tandem mass spectrometry (LC−MS/MS),[21−23] capillary electrophoresis (CE),[24] and enzyme-linked immunosorbent assay (ELISA).[25−27] Despite the high sensitivity and specificity that can be achieved by GC, HPLC and CE methods, they require expensive instrumentation, extensive sample cleanup and highly skilled personnel. For screening purposes, the immunoassay is advantageous compared to complex instrumental methods because of its high throughput and rapid turnaround time. The ELISA is an efficient immunoassay that can be used for analysis of

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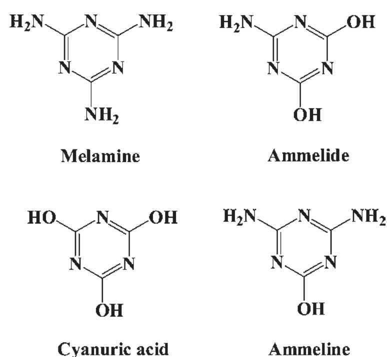

Figure 1. Chemical structures of melamine and related compounds.

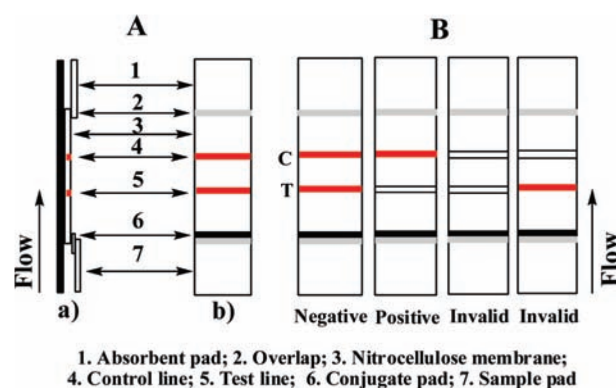

Figure 2. (A) Schematic description of a one step strip test: (a) side view cross section; and (b) top view. (B) Illustration of typical strip test results: C and T represent the control line and test line, respectively.

numerous samples, but it still requires labor-intensive operations including incubation, washing, and enzymatic reactions during the signal generation process. An immunogold chromatographic assay (IGCA) may be an alternative to the ELISA as a rapid screening method. Compared with the ELISA, the IGCA requires the least sample pretreatment, without the need for expensive equipment, and the results can be obtained within 3−10 min. However, until now, there has been no report concerned with using the method of IGCA for detection of MEL residues in biological matrices. Therefore, the aim of this study was to develop an immunochromatographic strip test to detect MEL residues in milk, milk products and animal feed.

MATERIALS AND METHODS

Reagents and Materials. MEL (purity ≥99.0%), bovine serum albumin (BSA), goat anti-mouse IgG and chlorauric acid ($HAuCl_4 \cdot 4H_2O$) were purchased from Sigma−Aldrich (St. Louis, MO, USA). Disodium hydrogen phosphate, polyethylene glycol (PEG), potassium carbonate, sodium chloride, trisodium citrate, Triton X-100, Tween 20 and other chemicals and solvents were purchased from Beijing Chemical Reagent Company (Beijing, China). *N,O*-Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) were obtained from Supelco/Sigma-Aldrich (Bellefonte, PA, USA). Purified water was obtained using a Milli-Q water purification system (Millipore, Bedford, MA). The nitrocellulose filter membrane (Millipore, HF13520s25) was purchased from Millipore Corporation (Millipore, Bedford, MA). The sample pad (CH37K), and the absorbance pad (SB08) were supplied by Shanghai Liangxin Co. Ltd. (Shanghai, China).

Apparatus. The NanoDrop ND-1000 spectrophotometer was purchased from Gene Company Limited (Hong Kong, China). The HQ-60-II vortex mixer was obtained from Beijing North TZ-Biotechnology Development Co. (Beijing, China), and the 5804R centrifuge was purchased from Eppendorf AG (Hamburg, Germany). The ZX1000 Dispensing Platform and the CM4000 Guillotine Cutting Module used to prepare the test strips were purchased from BioDot Inc. (Irvine, CA, USA).

Preparation of Capture Agents and Antibody. The coating antigen was prepared by conjugating MEL to BSA using the mixed anhydride method as described by Liu et al.,[27] with some modifications. Briefly, MEL was reacted with succinic anhydride to yield the MEL hapten. Sequentially, 1 M sodium bicarbonate (5 mL) containing 20 mg of the MEL hapten, dioxane (3 mL), triethylamine (200 μL) and isobutyl chloroformate (50 μL) were added into a 20 mL glass reaction vessel. The mixture was constantly stirred for 30 min at 4 °C. The above solution was then added dropwise to 100 mg of BSA in 5 mL of 1 M sodium bicarbonate and gently stirred for 12 h at 4 °C, resulting in the plate coating antigen, MEL−BSA.

The anti-MEL monoclonal antibody (mAb) was obtained from WDWK Biotech Co. (Beijing, China). The mAb was dialyzed against 0.005 M NaCl at 4 °C for 3 days, and then dialyzed against distilled water for 24 h. The concentration of purified antibody was determined with the spectrophotometer at 280 nm and diluted to 0.1 mg/mL with deionized water and stored at −20 °C until needed in this investigation.

Measurement of Cross-Reactivity. To evaluate the specificity of the mAb, MEL and several structurally related compounds including cyanuric acid, ammeline and ammelide (Figure 1) were tested for cross-reactivity using the competitive indirect ELISA (ciELISA) described by Yin et al.[25] The values for percent cross-reactivity (CR) were calculated as follows:

$$\mathrm{CR}\ (\%) = [\mathrm{IC}_{50}(\mathrm{MEL})/\mathrm{IC}_{50}(\mathrm{compounds})] \times 100$$

Preparation of Colloidal Gold. Colloidal gold was prepared as follows:[28] 100 mL of a 0.01% chlorauric acid solution was heated to boiling under constant stirring, and then 2.0 mL of 1% trisodium citrate was immediately added. The reaction was boiled for another 15 min, and the solution was then cooled and reconstituted to the initial volume by adding deionized water.

Preparation of Detection Reagents. The optimal pH and antibody concentration required to achieve the best sensitivity were determined by checkerboard titration prior to conjugation with colloidal gold. With gentle stirring, the colloidal gold solution (10 mL) was adjusted to pH 7.0 with 0.1 M K_2CO_3, and then 500 μL of the purified anti-MEL mAb (0.1 mg/mL) was added dropwise. After a 10 min incubation at room temperature, 3 mL of 5% BSA was added. Following another 10 min incubation, the solution was centrifuged at 18000*g* for 30 min, and the red gold-labeled mAb precipitate was resuspended with 2 mL of dilution buffer (0.02 M phosphate buffer (PB), containing 5% sucrose, 1% BSA and 0.5% Tween 20, pH 7.4) and stored at 4 °C until needed in this investigation.

Immobilization of Capture Reagents. The MEL−BSA antigen and goat anti-mouse IgG were separately diluted to 0.5 mg/mL with coating buffer (0.02 M PB, pH 7.4), and then sprayed onto the nitrocellulose (NC) membrane by the ZX1000 dispensing platform resulting in the test line and control line, respectively. The sprayed volumes were 0.06 μL per mm for the test line and 0.08 μL per mm for the control line. The NC membrane was then dried for 2 h at 37 °C and stored under dry conditions at room temperature until needed in this investigation.

Assembly of the Strip Test Components. The strip assembly procedure was similar to that described by Chen et al.[29] with some

modifications. Briefly, the NC membrane coated with capture reagents was pasted on the center of the back plate, and the conjugate pad (glass fiber) coated with detection reagent was pasted on the back plate by overlapping a 2 mm section with the NC membrane. The sample pad was pasted with one end overlapping the conjugate pad. The absorbent pad was pasted on the other side of the back plate by also overlapping vha 2 mm section with the NC membrane. Then the whole assembled plate was cut into 4 mm width strips and stored under dry conditions at room temperature.[29] A schematic description of the colloidal gold-based one step strip is illustrated in Figure 2.

Test Procedure. About 6−8 drops (about 120−160 μL) of standard solution or sample extract were added onto the sample pad and allowed to flow to the other end of the membrane strip. The result could be read by visual inspection after 3−10 min. If MEL was absent in the sample, the detection reagent would then be trapped by the capture reagent to form a visible test line. If MEL was present in the sample, then it would compete with the capture reagent for the limited amount of detection reagent. When enough analyte was present, it would then prevent the detection reagent from binding the capture reagent, and the test line signal would decrease to a nonvisible line and the results would be positive. When the test procedure was properly performed, the control line was always visible.

Sample Pretreatment. In this study, samples include raw milk, milk products (milk drinks, milk powder, cheese, yogurt, condensed milk) and animal feed. The pretreatment methods used for the different samples are described below.

A. Sample Pretreatment for Raw Milk, Milk Drinks, Yogurt and Condensed Milk. For raw milk, 1 mL of sample was diluted with 1 mL of sample diluent (0.02 M phosphate buffered saline (PBS), containing 0.5% Tween 20, pH 7.4) prior to analysis. For milk drinks and yogurt, 1 mL of sample was diluted with 3 mL of sample diluent prior to analysis, and for condensed milk, 1 mL of sample was diluted with 7 mL of sample diluent prior to analysis.

B. Sample Pretreatment for Milk Powder. Milk powder (1 g) was weighed into a 50 mL polypropylene centrifuge tube with 10 mL of deionized water and vortexed for 2 min. The mixed milk sample (1 mL) was transferred to a 10 mL polypropylene centrifuge tube, where it was diluted with 1 mL of sample diluent, and the sample was ready for analysis.

C. Sample Pretreatment for Cheese. One gram of cheese was weighed into a 10 mL polypropylene centrifuge tube, 2 mL of methanol/water (2:3, v/v) was added and the mixture was vortexed for 2 min. The mixture was centrifuged at 7500*g* for 5 min, then 100 μL of supernatant was transferred to a 2 mL polypropylene centrifuge tube, and 300 μL of sample diluent was added and mixed for 30 s. Since cheese is acidic, the pH of the mixture was adjusted to 7.0 with 1 M NaOH prior to analysis.

D. Sample Pretreatment for Animal Feeds. One gram of animal feed (premix feed, complete feed, concentrate feed and fish meal) was finely ground and weighed into a 10 mL polypropylene centrifuge tube, 2 mL of methanol/water (2:3, v/v) was added, and the mixture was vortexed for 2 min. The mixture was centrifuged at 7500*g* for 5 min, then 100 μL of supernatant was transferred to a 2 mL polypropylene centrifuge tube, 300 μL of sample diluent was added and mixed, and the solution was ready for analysis.

Analysis of Spiked Samples. The various types of samples to be analyzed were spiked with the MEL standard solution (10 μg/mL, prepared in 0.02 M PB, pH 7.4). The final MEL concentrations were as follows: 0.01, 0.03, 0.05, and 0.1 μg/mL in raw milk; 1.0, 1.5, 2.0, and 2.5 g/mL in milk drinks, yogurt, and condensed milk; 0.5, 0.8, 1.0, and 1.2 μg/g in milk powder; 1.0, 1.5, 2.0, and 2.5 μg/g in cheese and animal feeds. Pretreatment of these samples was conducted according to the procedures previously described. For each spiked level of each sample, five replicates ($n = 5$) were analyzed for one assay, and three assays were repeated over 3 consecutive days. The spiked samples were analyzed to estimate the repeatability and the detection limit.

Table 1. Relationship of Colors, Maximum Absorption Wavelength and Diameter Sizes of Colloidal Gold with Trisodium Citrate Concentrations

trisodium citrate (mL)	color of colloidal gold	max absorption wavelength (nm)	colloidal gold diam (nm)
1.0	purple	527	31.7
1.6	dark red	520	19.6
2.0	bright red	518	15.3
2.5	orange-red	517	12.2
3.5	orange-red	517	12.1
5.0	orange-red	517	11.8

GC−MS Analysis. To validate the strip test, MEL-spiked raw milk samples were analyzed by a GC−MS method. The GC−MS system included an Agilent GC 6890N instrument with the 7683 autosampler, a 5973 MS detector, and an HP-5MS 30 m × 0.25 mm i.d., 0.25 μm film thickness column (Agilent, CA, USA). The transfer line, ion source, and quadrupole analyzer temperatures were maintained at 280, 230, and 160 °C, respectively. The MS detector was operated in the electron impact (EI) ionization mode. Data was acquired in the selected ion monitoring (SIM) mode for the following ions: m/z 99, 171, 327, and 342.

The pretreatment of samples for GC−MS analysis followed the method described by Xia et al.[30] Briefly, 5 g of raw milk was weighed into a 50 mL polypropylene centrifuge tube, and 25 mL of 1% trichloroacetic acid (TCA) was added and vortexed for 30 s. After adding 15 mL of 1% TCA, the mixture was ultrasonically extracted for 15 min, then 2 mL of a lead acetate solution (22 g/L) was added, and the volume was adjusted to 50 mL with 1% TCA. The mixture was vortexed for 1 min prior to centrifugation at 6000*g* for 10 min. The supernatant (5 mL) was loaded onto an Oasis MCX cartridge for purification. Finally, MEL was eluted with 3 mL of a 5% ammonia/methanol (v/v) solution. The collected eluate was evaporated to dryness under a nitrogen stream in a 50 °C water bath and then reconstituted with 600 μL of pyridine and 200 μL of a solution of BSTFA + 1% TMCS. The tightly closed tubes were incubated in the oven for 30 min at 70 °C, and the reaction products were subjected to GC−MS analysis.

Blind Samples of Raw Milk. The 52 blind samples of raw milk were supplied by the National Institute of Metrology, Beijing, P. R. China, and all samples were analyzed by both the strip test method and GC−MS for confirmation.

RESULTS AND DISCUSSION

Optimization of the Immunochromatographic Strip Test. To evaluate the effect of colloidal gold particle size on sensitivity of the strip test, several diameter sizes of colloidal gold particles were prepared by adding 1.0, 1.6, 2.0, 2.5, 3.5, or 5.0 mL of 1% trisodium citrate into 100 mL of 0.01% chlorauric acid. As the amount of trisodium citrate increased, the color of colloidal gold changed from purple to orange-red (Table 1). This result was the same as that described by Zhou et al.[28] In addition, the diameter sizes were determined by a transmission electron microscope and calculated to be 31.7, 19.6, 15.3, 12.2, 12.1, and 11.8 nm (Table 1). The highest sensitivity and stability and the best color development were obtained by using colloidal gold with a diameter of 15.3 nm. This result was different from that obtained in previous studies.[29,31,32] A diameter size for colloidal gold of 15.3 nm also was the most suitable for

Table 2. Cross-Reactivity of the MEL MAb with Structurally Related Compounds

compound	IC_{50} (ng/mL)	cross-reactivity (%)
melamine	19.2	100
cyanuric acid	>2000	<1
ammelide	>2000	<1
ammeline	>2000	<1

Table 3. Results of the Strip Test Assay Sensitivity for MEL Concentrations in Raw Milk

spiked level of MEL (μg/mL)	strip test[a] (n = 5)		
	assay 1	assay 2	assay 3
0	− − − − −	− − − − −	− − − − −
0.01	− − − − −	− − − − −	− − − − −
0.03	±±±±±	±±±±±	±±±±±
0.05	+++++	+++++	+++++
0.10	+++++	+++++	+++++

[a] −, absence of MEL; +, presence of MEL; ±, weakly positive.

conjugation with the anti-MEL mAb and, therefore, was selected for further experimentation.

The previous studies[29,31,32] also reported that the amount of antibody and pH are very important during the conjugation of colloidal gold with an antibody (Ab). In general, the optimum pH value for gold−Ab conjugation is at the isoelectric point (p*I*) of the antibody or 0.5 pH unit higher.[33] The optimal pH and optimal amount of antibody for the conjugation reaction can be determined by measuring the differential absorbance according to the method described by Paek et al.[33] In our study, it was found that pH 6 appeared optimal and the optimal amount of mAb was 10 μg/mL of colloidal gold. However, at high concentrations of MEL (even 1 mg/mL), MEL would not completely bind the limited amount of detection reagents. The results indicated that these conditions were not conducive to produce good MEL strip test sensitivity. Therefore, a two-dimensional titration assay was used to obtain the best sensitivity of the strip test. Various pH values (6.0, 7.0, 8.0, and 9.0) and various amounts of the MEL mAb (2.5, 5, 10, 20 μg/mL colloidal gold) were screened for the optimum combination. The results demonstrated that the optimal pH value was 7.0 and the optimum amount of mAb was 5 μg/mL of colloidal gold.

Subsequently, three coating buffer systems were evaluated and compared to immobilize MEL−BSA and goat anti-mouse IgG on the NC membrane, which included carbonate buffer (CB, 0.05 M, pH 9.6), PB (0.02 M, pH 7.4), and PBS (0.02 M, pH 7.4). The sensitivity and color development of the strip test were better using CB and PB than when using PBS. However, the stability of the strip test using PB was better than when using CB. Previous studies[28,29,31] reported that the best result was obtained using PBS or CB. Perhaps different coating antigens require different conditions to adequately bind the NC membrane.

The time required for drying the NC membrane used to immobilize the capture reagents was evaluated from 30 min to overnight at 37 °C. As a result, capture reagents incubated for 2 h at 37 °C demonstrated high stability and the best color development. Thus, 2 h at 37 °C was selected for the drying conditions. A series of experiments tested the need for blocking the NC membrane after the coating step by using two different blocking buffers (0.1% BSA and 0.1% casein). The results showed that the blocking process had no significant effect on assay sensitivity compared with the unblocked NC membrane. Therefore, after immobilizing the capture reagents the NC membrane was not blocked.

Table 4. Results of the Strip Test Assay Sensitivity for MEL Concentrations in Various Sample Types (Milk Drinks, Yogurt, Condensed Milk, Cheese and Animal Feed)[a]

spiked level of MEL (μg/mL or μg/g)	strip test[b] (n = 5)		
	assay 1	assay 2	assay 3
0	− − − − −	− − − − −	− − − − −
1.0	− − − − −	− − − − −	− − − − −
1.5	±±±±±	±±±±±	±±±±±
2.0	+++++	+++++	+++++
2.5	+++++	+++++	+++++

[a] Results for all sample types were identical. [b] −, absence of MEL; +, presence of MEL; ±, weakly positive.

The effect of dilution buffer on the gold−mAb complex was studied. The influence of surfactant (Tween 20 and Triton X-100) on assay performance was evaluated first. It was found that low concentrations of surfactant would not allow the sample solution to flow favorably, and high concentrations of surfactant increased the sample solution flow rate too rapidly, resulting in inadequate antibody/antigen response. Following trial and error testing, the use of 0.5% (v/v) Tween 20 was determined to result in the highest sensitivity and the best color development. BSA is often used as a protective reagent during dilution of the gold−Ab complex; it can also intensify color development, eliminate the background, and speed up the sample solution flow rate. Although BSA has a strong role in enhancing color development, high levels of BSA can reduce the sensitivity of the strip test. Therefore, 1% BSA was used in the dilution buffer. The effect of sucrose was evaluated. Sucrose can promote the release of the gold−Ab complex from the conjugate pad and improve the stability of the strip, but high concentrations of sucrose also can reduce the sensitivity. Thus, 5% sucrose was chosen.

Finally, assay sensitivity was investigated with respect to the pretreatment of the conjugate pad and sample pad. It was found that when the conjugate pad and sample pad were pretreated with dilution buffer containing the surfactants, Tween 20 or PEG, and the protein, BSA, the flow rate of the sample solution was accelerated, the background was eliminated, the color development was intensified for the negative sample, and the sensitivity and stability of the strip test were improved. Therefore, the pretreatment solution consisting of 0.02 M PB, containing 2% sucrose, 0.5% BSA and 0.5% Tween 20, pH 7.4 was selected.

Specificity of the Strip Test. The results of cross-reactivity studies demonstrated that the MEL mAb had negligible cross-reactivity with cyanuric acid, ammeline and ammelide (<1%) (Table 2), indicating that the mAb has high specificity. The obtained results were consistent with other research.[25] These results showed that the chemical groups on the triazine ring (Figure 1) play an important role in antibody binding.

Table 5. Results of the Strip Test Assay Sensitivity for MEL Concentrations in Milk Powder

spiked level of MEL (μg/g)	strip test[a] ($n = 5$)		
	assay 1	assay 2	assay 3
0	− − − − −	− − − − −	− − − − −
0.5	− − − − −	− − − − −	− − − − −
0.8	± ± ± ± ±	± ± ± ± ±	± ± ± ± ±
1.0	+ + + + +	+ + + + +	+ + + + +
1.2	+ + + + +	+ + + + +	+ + + + +

[a] −, absence of MEL; +, presence of MEL; ±, weakly positive.

Figure 3. Strip test for the detection of MEL in raw milk. The spiked levels used were 0, 0.01, 0.03, 0.05, and 0.1 μg/mL from left to right. The level of 0.05 μg/mL MEL in raw milk causes complete disappearance of the test line.

Analysis of Spiked Samples. In this study, the matrix effect was determined for milk, milk products and animal feed. The sample pretreatment used prior to conducting the strip test is very simple and rapid. For liquid milk (raw milk, milk drinks, yogurt and condensed milk), samples were prepared by performing a simple dilution step with the sample diluent. To minimize the matrix interference, the dilution factor was adjusted for the different liquid milk products. Milk powder was first dissolved in deionized water, and then diluted with the sample diluent prior to analysis. Cheese and animal feeds were first mixed with a methanol/water solution to extract MEL, the supernatant that contained MEL was obtained by centrifugation, and then the samples were diluted using the sample diluent.

Samples from the three different matrix types were spiked with different levels of MEL to determine the detection limit of the strip test. In this study, the detection limit of the strip test was defined as the MEL concentration of sample solution that resulted in total invisibility of the test line. The results are summarized in Tables 3−5. The limit of detection was estimated to be 0.05 μg/mL in raw milk, since at this concentration there was a complete disappearance of the detection line (Figure 3). Compared with the ELISA study of Yin et al.,[25] this strip test was more sensitive and rapid. The limit of detection was estimated to be 2 μg/mL (or 2 μg/g) in milk drinks, yogurt, condensed milk, cheese and animal feed (Figure 4), and 1 μg/g in milk powder (Figure 5).

Figure 4. Strip test for the detection of MEL in animal feed (concentrate feed). The spiked levels used were 0, 1.0, 1.5, 2.0, and 2.5 μg/g from left to right. The level of 2.0 μg/g MEL in animal feed causes complete disappearance of the test line.

Figure 5. Strip test for the detection of MEL in milk powder. The spiked levels used were 0, 0.5, 0.8, 1.0, and 1.2 μg/g from left to right. The level of 1.0 μg/g MEL in milk powder causes complete disappearance of the test line.

Comparative Study between the Strip Test and GC−MS. GC−MS analysis was performed in parallel with the strip test as a confirmatory method for identification and quantitation of MEL in 52 blind raw milk samples. The raw milk samples were all analyzed by the strip test and confirmed by GC−MS. The limit of quantification of the GC−MS method was 0.005 μg/mL for raw milk. As shown in Table 6, the results of sample analysis using the strip test were in good agreement with the results obtained from GC−MS, indicating that the strip test we developed had good reliability, and the strip test gave neither false positive nor false negative results.

This is the first report of using an immunogold chromatographic assay to analyze for MEL in raw milk, milk products and

Table 6. Comparison of MEL Analyses Using GC−MS and the Strip Test in 52 Blind Raw Milk Samples

sample no.	GC−MS (ng/mL)	strip test[a] ($n = 5$)	sample no.	GC−MS (ng/mL)	strip test ($n = 5$)
426	0.0	− − − − −	272	51.2	+ + + + +
441	0.0	− − − − −	274	52.4	+ + + + +
450	0.0	− − − − −	280	48.8	+ + + + +
479	0.0	− − − − −	308	49.5	+ + + + +
415	0.0	− − − − −	123	96.0	+ + + + +
462	0.0	− − − − −	135	100.2	+ + + + +
473	0.0	− − − − −	137	97.5	+ + + + +
482	0.0	− − − − −	148	103.4	+ + + + +
456	9.8	− − − − −	184	105.6	+ + + + +
466	7.5	− − − − −	186	99.6	+ + + + +
469	11.5	− − − − −	193	100.7	+ + + + +
434	10.8	− − − − −	120	101.0	+ + + + +
459	8.9	− − − − −	126	98.4	+ + + + +
465	10.0	− − − − −	131	102.6	+ + + + +
206	33.6	± ± ± ± ±	146	103.8	+ + + + +
243	30.9	± ± ± ± ±	192	97.3	+ + + + +
291	31.7	± ± ± ± ±	200	95.1	+ + + + +
295	36.5	± ± ± ± ±	438	105.4	+ + + + +
128	28.4	± ± ± ± ±	229	2000.0	+ + + + +
248	32.2	± ± ± ± ±	241	2000.0	+ + + + +
285	29.8	± ± ± ± ±	250	2000.0	+ + + + +
319	34.4	± ± ± ± ±	257	2000.0	+ + + + +
262	55.7	+ + + + +	228	2000.0	+ + + + +
270	49.4	+ + + + +	232	2000.0	+ + + + +
271	50.5	+ + + + +	239	2000.0	+ + + + +
281	47.3	+ + + + +	240	2000.0	+ + + + +

[a] −, absence of MEL; +, presence of MEL; ±, weakly positive.

animal feed. Sample pretreatment was simple and rapid, and the results could be obtained within 3−10 min. The detection limit for each sample type meets the detection limit requirements of Australia, China, the Food and Drug Administration (FDA), and the EU. Therefore, the developed strip test is suitable for detecting MEL residues onsite in large numbers of raw milk, milk products and animal feed samples. Since this method provides only qualitative and semiquantitative results, the determined positive samples should be further confirmed by more sensitive methods such as HPLC, LC−MS/MS or GC−MS.

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ABBREVIATIONS USED

Ab, antibody; BSA, bovine serum albumin; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; CB, carbonate buffer; CE, capillary electrophoresis; CR, cross-reactivity; EI, electron impact; ELISA, enzyme-linked immunosorbent assay; EU, European Union; FDA, Food and Drug Administration; GC−MS, gas chromatography−mass spectrometry; HPLC, high-performance liquid chromatography; IGCA, immunogold chromatographic assay; LC−MS/MS, liquid chromatography−tandem mass spectrometry; MEL, melamine; TMCS, trimethylchlorosilane; MEL, melamine; MEL−BSA, melamine−bovine serum albumin; NC, nitrocellulose; NPN, nonprotein nitrogen; PB, phosphate buffer; PBS, phosphate buffered saline; PEG, polyethylene glycol; p*I*, isoelectric point; SIM, selected ion monitoring; TCA, trichloroacetic acid

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