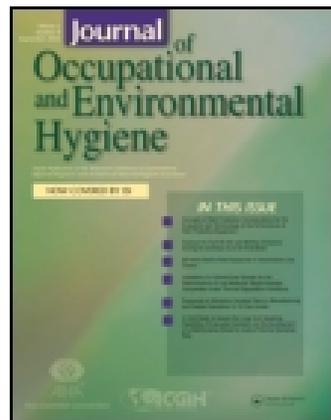


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Enhanced Performance of Methamphetamine Lateral Flow Cassettes Using an Electronic Lateral Flow Reader

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Surface contamination from methamphetamine in meth labs continues to be a problem. We had previously developed a lateral flow assay cassette for field detection of methamphetamine contamination that is commercially available and has been used by a number of groups to assess contamination. This cassette uses the complete disappearance of the test line as an end point for detection of 50 ng/100 cm² of methamphetamine contamination for surface sampling with cotton swabs. In the present study, we further evaluate the response of the cassettes using an electronic lateral flow reader to measure the intensities of the test and control lines. The cassettes were capable of detecting 0.25 ng/ml for calibration solutions. For 100 cm² ceramic tiles that were spiked with methamphetamine and wiped with cotton-tipped wooden swabs wetted in assay/sampling buffer, 1 ng/tile was detected using the reader. Semi-quantitative results can be produced over the range 0–10 ng/ml for calibration solutions and 0–25 ng/tile for spiked tiles using either a 4-parameter logistic fit of test line intensity versus concentration or spiked mass or the ratio of the control line to the test line intensity fit to concentration or spiked mass. Recovery from the tiles was determined to be about 30% using the fitted curves. Comparison of the control line to the test line was also examined as a possible visual detection end point and it was found that the control line became more intense than the test line at 0.5 to 1 ng/ml for calibration solutions or 1 to 2 ng/tile for spiked tiles. Thus the lateral flow cassettes for methamphetamine have the potential to produce more sensitive semi-quantitative results if an electronic lateral flow reader is used and can be more sensitive for detection if the comparison of the control line to the test line is used as the visual end point.

Keywords direct reading, lateral flow, methamphetamine

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INTRODUCTION

According to the United States Drug Enforcement Administration (DEA) discovery of clandestine metham-

phetamine laboratories peaked at 17,000 in 2003–2004 (http://www.justice.gov/dea/concern/map_lab_seizures.html). State and federal laws restricting availability of methamphetamine precursors, particularly pseudoephedrine or ephedrine, have led to initial decreases in clandestine laboratory discoveries or seizures.⁽¹⁾ However, thousands are still found each year. Small-scale methamphetamine laboratories supply approximately 20% of the U.S. methamphetamine supply,^(2,3) and this number is expected to increase.⁽⁴⁾

Residual contamination of clandestine methamphetamine laboratories represents a hazard to emergency response personnel, remediation workers, and the general public.^(5–7) Reducing risks for methamphetamine exposures involves awareness of surface contamination, especially the risks for contact transfer of methamphetamine to hands and other skin surfaces as the primary route. The National Institute for Occupational Safety and Health (NIOSH) has developed numerous methods for surface sampling and analysis to detect methamphetamine on surfaces (*NIOSH Manual of Analytical Methods*, Methods 9106, 9109, and 9111). The methods all use mass spectroscopy and isotopic dilution but differ in sample preparation and analysis. NMAM 9106 and 9109 are gas chromatography/mass spectroscopy (GC/MS) methods, and 9111 is a liquid chromatography/mass spectroscopy (LC/MS) method.

While laboratory methods are sensitive and accurate they do have shortcomings. Surface samples need to be collected, transported to the laboratory, and analyzed, a time-consuming process requiring specialized equipment and trained personnel. In light of this, NIOSH was contacted by law enforcement and public health agencies to develop rapid tests that could be used in the field with minimal training.

Previously we have described a method that uses a lateral flow assay cassette to detect methamphetamine contamination in real time in the field.⁽⁸⁾ This cassette is capable of detecting 50 ng/100 cm² methamphetamine surface contamination using complete disappearance of the test line as the end point with solutions produced by wiping the surface with cotton swabs. In the present study we explore the use of the same lateral

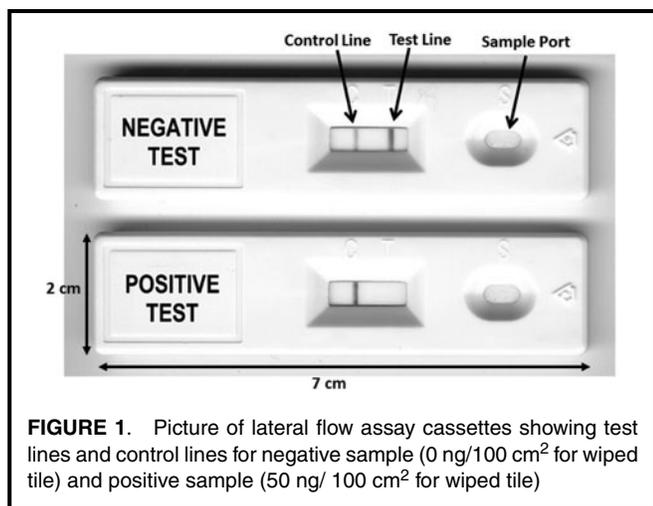


FIGURE 1. Picture of lateral flow assay cassettes showing test lines and control lines for negative sample (0 ng/100 cm² for wiped tile) and positive sample (50 ng/ 100 cm² for wiped tile)

flow assay cassettes with an electronic reader to see if this modification might allow more sensitive measurements of contamination with possible semi-quantitative evaluations.

METHODS AND MATERIALS

Description of Lateral Flow Assay Cassettes for Methamphetamine

The lateral flow cassettes shown in Figure 1 for positive and negative samples allow the onsite detection of methamphetamine contamination. They have been described previously.⁽⁸⁾ The cassettes consist of a sample port where the liquid sample in assay buffer is introduced and a membrane area with two lines: the test line and control line. The liquid sample carries reagents that are contained in the cassette to the membrane area using capillary flow. The test line has anti-methamphetamine antibodies that will bind a gold-labelled methamphetamine-bovine serum albumin conjugate resulting in a read color at the test line.

If there is methamphetamine present in the sample, it will compete with the gold-labelled methamphetamine-bovine serum albumin conjugate for binding to the test line so the test line becomes less intense with increasing concentrations of methamphetamine in solution. The control line uses a separate binding mechanism that is not affected by methamphetamine and it remains relatively constant with increasing methamphetamine concentration. The control line must be present for a valid test. The test line will completely disappear at some concentration of methamphetamine in solution. These particular cassettes for methamphetamine have been designed to give complete disappearance of the test line as the end point for visual detection of 50 ng/100 cm² of methamphetamine on a surface that is sampled with cotton-tipped swabs.

Reagents and Apparatus

The methamphetamine lateral flow assay cassettes were made by Arista Biologicals (Allentown, PA) and are identical to the ones sold in the MethChek 50 kit (SKC Inc. Eighty Four,

PA). The assay/sampling buffer was phosphate buffered saline (PBS) (Sigma product no. 3563)(Sigma-Aldrich, St. Louis, MO) with 0.1% W/V Triton X-100 (Mallinckrodt Specialty Chemicals Co, Paris, KY). The methamphetamine stock standard which was 100,000 ng/ml was made by dissolving a preweighed amount of methamphetamine in methanol. The tiles used for determining surface sampling efficiency were 4" × 4" (100 cm²) white ceramic bathroom tiles. Swabs for wiping tiles were Puritan 806-WC (Hardwood Products Co., Guilford, ME). The lateral flow cassette reader was a Hamamatsu C10066 Immunochromato Reader (Hamamatsu, Iwata City, Japan) used with the supplied software.

Calibration Solution Preparation and Response

Calibration solutions over a range of concentrations (0, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 40.0 ng/ml) were prepared by serial dilution of methamphetamine stock standard in assay/sampling buffer. Three drops of the solutions were applied to the methamphetamine lateral flow cassettes with the dropper supplied with the cassettes and the intensity of the test line and control lines were determined with the Hamamatsu reader at 5 min, 10 min, and 15 min intervals after applying the solutions. The response was also evaluated visually by assessing the presence or absence of the test line and by comparing the intensity of the test line and control line.

Tile Spiking and Surface Sampling from Tiles

Spiking solutions were prepared by serial dilution of the methamphetamine stock standard in methanol. The spiking solutions had concentrations such that spiking tiles with 50 ul would produce the desired surface loadings of 0, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, and 100 ng/tile. The tiles were spiked with the proper solution and allowed to dry. Each 100 cm² tile was completely wiped with a cotton swab that was wetted in a vial containing 1 mL of sampling/assay buffer (PBS-0.1% Triton X-100 (w/v)). The tiles were wiped, first in an up-and-down direction, then in a sideways direction, and finally the up-and-down wiping direction was repeated. The swab was then returned to the vial containing sampling/assay buffer and the vial was agitated vigorously for 2 min. The resulting solutions were applied to the lateral flow cassettes using the same procedure that was used with the calibration solutions and the response was evaluated in the same way.

Evaluation of Response

The response of the assay cassettes was evaluated at each time interval in a number of ways. The intensity of the test line measured by the Hamamatsu reader was evaluated against calibration solution concentration or tile surface loading. The value of %B/Bo for the test line (where B = the test line intensity for each individual calibration solution or wipe solution and Bo = test line intensity for the corresponding blank) was calculated and plotted against calibration solution concentration or tile surface loading. Standard curves were constructed for calibration solutions and wipe solutions from four-parameter logistic-log fits (4-PL, SigmaPlot, SPSS,

Chicago, IL) of %B/Bo for the test line as a function of calibration solution concentration or tile surface loading. Assessment of the “goodness of fit” and the dynamic ranges of the assays were investigated by evaluating the fit of the standards data to the 4-PL model by “standards recovery,”⁽⁹⁾ calculated by evaluating interpolated results from each 4-PL fit (observed concentration or mass) and comparing it to the actual concentrations of calibration solutions or the spiked mass (the expected concentration or mass) using the following relationship: %recovery = 100 × (observed concentration from 4-PL fit of data / expected concentration). The resultant data were analyzed for linearity by linear regression.

The ratio of the control line intensity to the test line intensity (C/T) at each time point for the calibration solutions and tile spiked tile solutions was plotted directly against concentration or spiked mass.

The recovery was determined by computing the concentration of the solutions from wiped tiles using the curves from the calibration solutions and comparing these values to the concentration calculated from the spiked masses of methamphetamine assuming 100% recovery. The recovered mass was fitted to the spiked mass using least squares fit and the recovery from the surface was evaluated by the slope of the recovered mass curve. This was done with both the 4-PL and the C/T fitted lines.

Visual interpretation involved assessment of the presence (P) or absence (A) of the test line. If the test line was barely visible and had no color, it was judged as a ghost line (G). The visual assessment also compared the intensity of the test line and control line and if the control line was more intense it was rated (C) or if the test line was more intense it was rated (T). If the lines were judged as equal then (=) was used. If the lines were judged as close to equal but the test line was slightly brighter then (T =) was used and if control line was slightly more intense then (C =) was used.

RESULTS

Response Curves

The response curves for the calibration solutions and spiked tile solutions were the average of three separate runs done on three separate days. Figure 2A shows the response of the cassettes for calibration solutions presented as %B/Bo for the test line as a function of solution concentration. The %B/Bo is about 80% at 0.25 ng/ml and reaches less than 3% at 40 ng/ml. This curve was fitted with the 4-PL model and the fit was used to calculate observed concentration which is plotted against expected concentration in Figure 2B, showing a good fit over the range 0–10 ng/ml. Figure 2C shows the response for the cassettes for solutions from spiked tiles presented as %B/Bo for the test line as a function mass spiked on the tile. The %B/Bo is about 80% at 1 ng/tile and reaches less than 3% at 100 ng/tile. This curve was fitted with the 4-PL model and the fit was used to calculate observed mass which is plotted against expected mass in Figure 2D.

Figure 3A shows the C/T ratio as a function of concentration for the lateral flow cassettes developed with the calibration solutions. Figure 3B shows the C/T ratio plotted against spiked mass for cassettes developed with solutions from the spiked tiles. The lateral flow cassettes showed the same response after 5, 10, and 15 min so they can be used after 5 min of development.

Recovery from Spiked Tiles

The recovery from spiked tiles was calculated using the both the 4-PL fit for %B/Bo curve and C/T ratio curve from the calibration solutions to calculate the concentration of solutions from wiping the spiked tiles. Figure 4A shows the recovery curve using the solution data from the 4-PL plots and Figure 4B shows the recovery curve for C/T ratio.

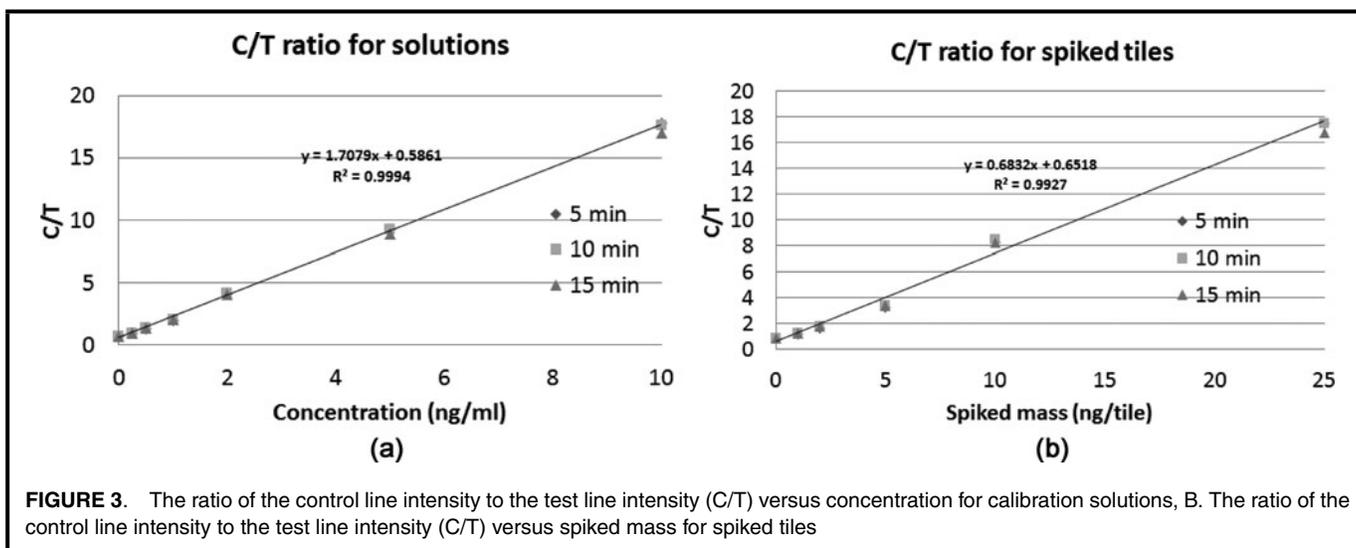
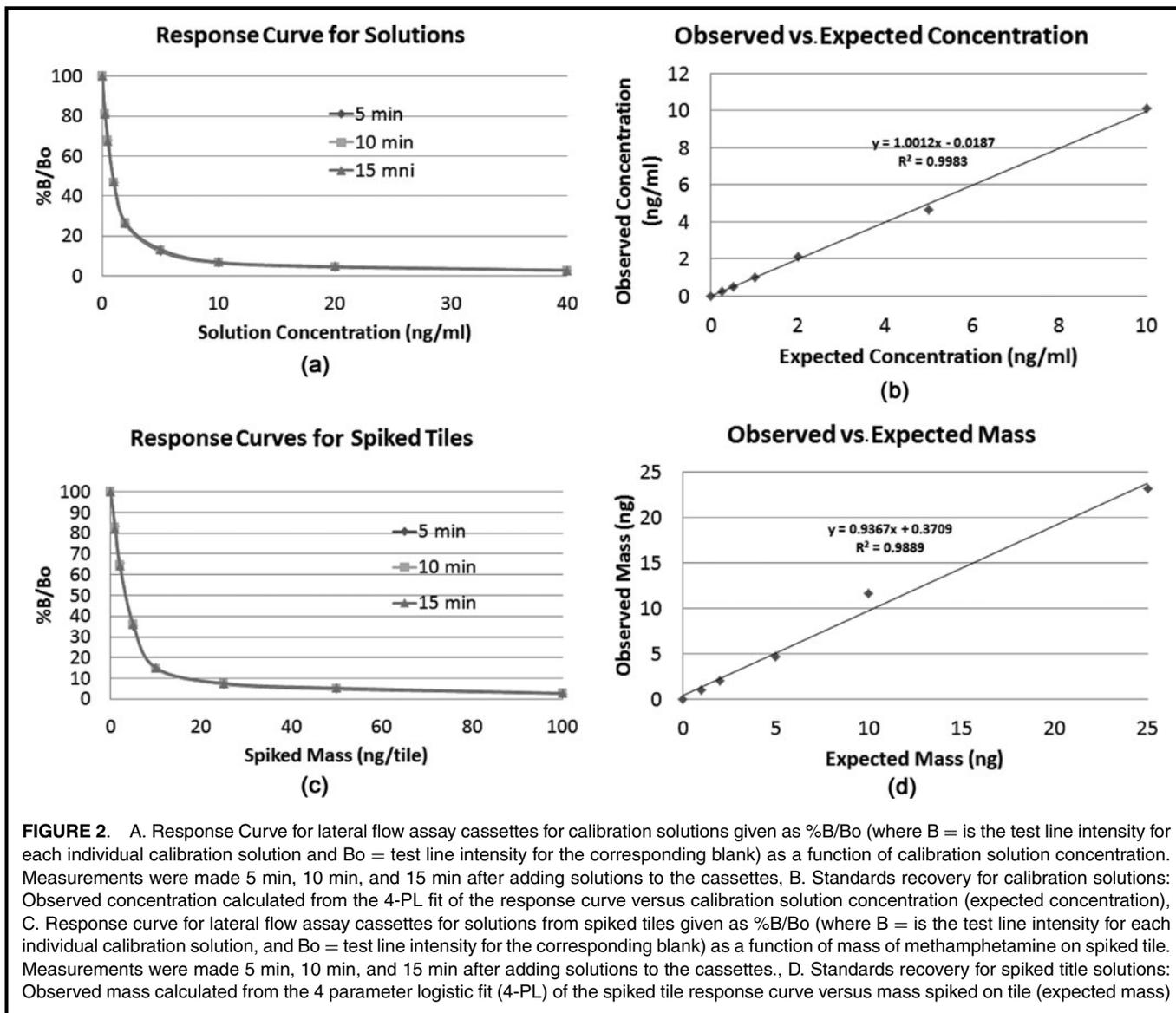
Visual Interpretation

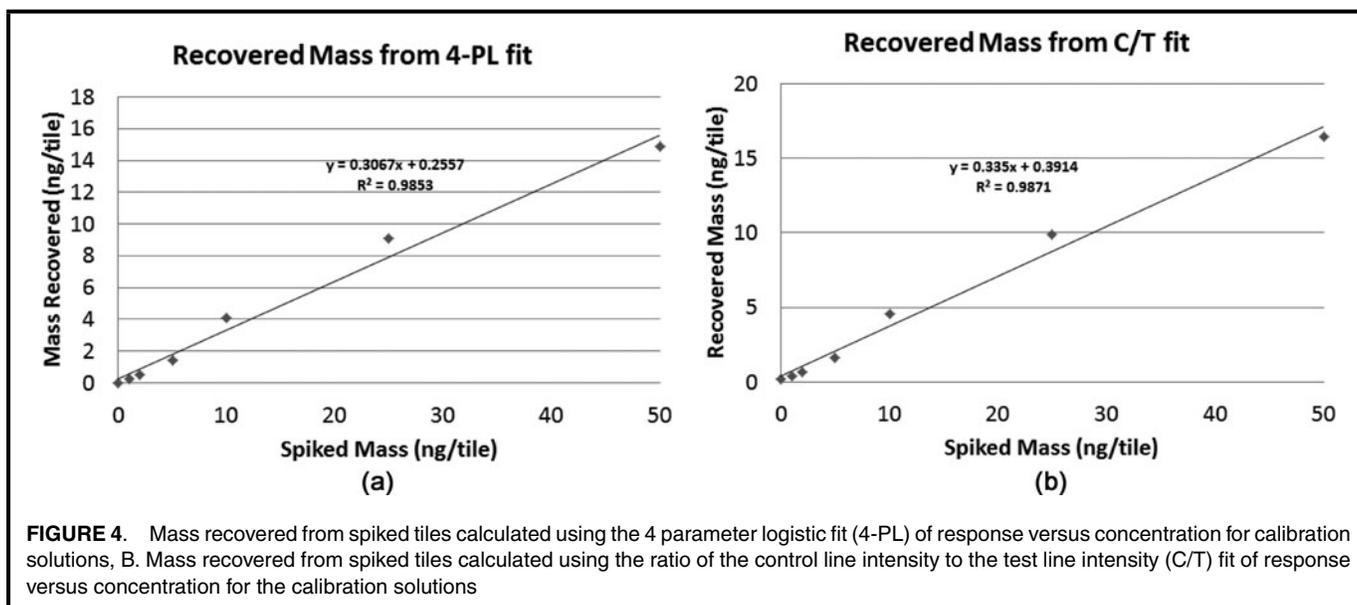
Table I shows the most intense line and absence or presence of the test line data for the cassettes used with calibration solutions and solutions from the wiped tiles. A range is given since there were three experiments done on three different days but there were similar results from day to day.

DISCUSSION

The data shown indicate that the cassettes are capable of detecting 0.25 ng/ml for calibration solutions and 1 ng/tile for spiked tiles using the electronic reader. They are capable of semi-quantitative results over the range 0–10 ng/ml for calibration solutions and 0–25 ng/tile for spiked tiles using either a 4-PL fit of %B/Bo versus concentration or using the C/T ratio fit to concentration. The advantage of the C/T ratio is that it is simple to calculate and also might provide some compensation for variation in the response of the cassettes since the test line and control line would be expected to vary in the same way. The calculated recovery from the tiles using the fitted calibration solutions curves to calculate the concentration of the solutions from the tile wipes gave about 30% recovery from the spiked tiles. This agrees with values from a previously study where recovery was determined by fluorescence covalent microbead immunosorbent assay.⁽¹⁰⁾ Electronic readers for lateral flow assays are easy to use and therefore can be used with minimal training. Although there is an expense associated with purchase of the reader, this cost is diminishing as more portable readers become available for use in clinical point-of-care applications. Several of these readers use smart phone technology.

For visual interpretation of response, this study agrees with the previous study⁽¹⁰⁾ and specifications for the cassettes. Complete disappearance of the test line was observed at 50 ng/tile for the tiles wiped with cotton swabs since all cassettes gave either absence of the test line (A) or a ghost line (G) at this level. Since recovery from the tiles was about 30% and 30% of 50 ng/tile is close to 20 ng/ml for the resulting solution, this agrees with 20 ng/ml for complete disappearance of the test line with calibration solutions. Using the comparison



**TABLE I. Methamphetamine Cassette Performance Using Visual Interpretation**

Cal solutions Concentration (ng/ml)	more intense line-control (C) or test (T)			Cal solutions Concentration (ng/ml)	Test line present (P) or absent (A)		
	5 min	10 min	15 min		5 min	10 min	15 min
0	T	T	T	0	P	P	P
0.25	T =	T = to T	T = to =	0.25	P	P	P
0.5	C =	C = to C	C	0.5	P	P	P
1	C	C	C	1	P	P	P
2	C	C	C	2	P	P	P
5	C	C	C	5	P	P	P
10	C	C	C	10	P to G	P to G	P to G
20	C	C	C	20	G to A	G to A	G to A
40	C	C	C	40	A	A	A

Tile solutions spiked mass (ng/tile)	more intense line-control (C) or test (T)			Tile solutions spiked mass (ng/tile)	Test line present (P) or absent (A)		
	5 min	10 min	15 min		5 min	10 min	15 min
0	T = to T	T = to T	T = to T	0	P	P	P
1	C =	C =	C = to C	1	P	P	P
2	C	C	C	2	P	P	P
5	C	C	C	5	P	P	P
10	C	C	C	10	P	P	P
25	C	C	C	25	P to G	P to G	P to G
50	C	C	C	50	G to A	G to A	G to A
100	C	C	C	100	A	A	A

Note: Cassettes were developed with calibration solutions and solutions from spiked tiles. The results were evaluated by assessing visually which was the more intense line and from the presence or absence of the test line. If the test line was more intense then T was used and if the control line was more intense then C was used. If lines were judged as equal then = was used. If the lines were close to equal but the test line was slightly brighter, then T = was used. If the lines were almost equal but the control was judged slightly brighter, then C = was used. If the test line was present then P was used; if the test line was absent then A was used, if the test line was barely visible with no color then G for ghost line was used. A range is given at some time points since the results are from three experiments.

of the control line to the test line, much lower levels could be detected since the control line (C) was more intense than the test line (T) at 0.5 to 1 ng/ml for calibration solutions or 1 to 2 ng/tile for spiked tiles.

States have different requirements for levels of methamphetamine contamination after cleanup generally ranging from 50 ng/100 cm² to 1500 ng/100 cm². The range of this method using the electronic reader is 0–25 ng/100 cm² for semi-quantitative results using 1 ml of assay/sampling buffer for extraction of the swab after surface sampling. To extend the range to higher levels, increased volumes of the assay/sampling buffer for sampling swab extraction could be employed (2 ml for 50 ng/100 cm² up to 100 ml for 1500 ng/100 cm²). This is essentially the same approach that SKC uses to produce kits capable of detecting 50 ng/100 cm², 100 ng/100cm², 500 ng/100 cm², and 1500 ng/100 cm² employing the same methamphetamine lateral flow cassette that was studied in this article and using complete disappearance of the test line as the visual endpoint. One area for further study would be the evaluation of recovery from multiple types of surfaces in addition to the ceramic tiles used in this study.

CONCLUSION

The lateral flow cassettes for methamphetamine have the potential to produce more sensitive semi-quantitative results if an electronic lateral flow reader is used and can be more sensitive for detection if the comparison of the control line to the test line is used as the visual end point.

DISCLAIMER

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