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Detection of 5-fluorouracil surface contamination in near real time

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Abstract

Objectives: Contamination of workplace surfaces by antineoplastic drugs presents an exposure risk for healthcare workers. Traditional instrumental methods to detect contamination such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) are sensitive and accurate but expensive and incapable of producing results in real time. This limits their utility in preventing worker exposure. We are currently developing monitors based on lateral flow immunoassay that can detect drug contamination in near real time. In this report, we describe the laboratory performance of a 5-fluorouracil (5-FU) monitor.

Methods: The monitor was evaluated by spiking ceramic, vinyl, composite, stainless steel, and glass surfaces of 100 cm^2 area with 5-FU masses of 0, 5, 10, 25, 50, and 100 ng. The surface was sampled with a wetted cotton swab, the swab was extracted with buffer, and the resulting solution was applied to a lateral flow monitor. Two ways of evaluating the response of these monitors were used: an electronic method where a lateral flow reader was used for measuring line intensities, and a visual method where the intensity of the test line was visually compared to the control line.

Results: The 5-FU monitor is capable of detecting 10 ng/100 cm^2 (0.1 ng/cm^2) using the electronic reader and 25 ng/100 cm^2 (0.25 ng/cm^2) using the visual comparison method for the surfaces studied. The response of the monitors was compared to LC-MS/MS results for the same samples for validation and there was good correlation of the two methods but some differences in absolute response, especially at higher spiking levels for the surface samples.

Keywords

Antineoplastic drugs, lateral flow, direct reading

Introduction

Many antineoplastic drugs have known carcinogenic, mutagenic, and adverse reproductive effects.[1,2] Currently, an estimated 8 million US health care workers are potentially exposed to antineoplastic drugs.[3] A number of studies have documented workplace contamination by antineoplastic drugs and have resulted in the development of safe handling procedures. NIOSH has developed an Alert where information concerning the effects of exposure is given, processes producing exposure are described, and procedures for lowering exposure are presented.[1] However, recent studies have shown that despite following recommended safe handling practices, workplace contamination with antineoplastic drugs in pharmacy and nursing areas continues to occur.[4,5]

Analytical techniques for measurement of surface contamination by antineoplastic drugs such as LC-MS/MS[6] are sensitive, specific and accurate, but preparation of samples for analysis is time consuming, the initial equipment investment is expensive, and such

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methods cannot provide immediate information about workplace contamination to help in controlling exposures. A direct reading technique was recently developed by our group to evaluate methamphetamine contamination based on surface wiping and lateral flow immunoassay.[7] Because of the success of this technique it was decided to explore the use of a similar technique for antineoplastic drugs. In this paper, the laboratory performance of a 5-fluorouracil (5-FU) surface contamination detection technique based on surface wiping and lateral flow immunoassay is described.

Experimental

Reagents and monitor

Since the 5-FU lateral flow drug monitor is being considered for commercial development, this paper will present performance data only and details concerning the development of the monitors will be provided at a future date. Cotton swabs were Puritan model 806-WC (Puritan, Guilford, ME). 5-fluorouracil (5-FU $\geq$ 99%, product number F6627-1G) and polyoxyethylenesorbitan monolaurate (Tween 20, product number P-1379) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Concentrated ammonium hydroxide (product A669-500) was from Fisher Scientific (Fair Lawn, NJ, USA).

Principle of operation

The 5-FU monitors employ competitive lateral flow immunoassay to detect the presence of 5-FU on surfaces. The principle of the monitors is shown in Figure 1. The monitors have an anti-5-FU antibody conjugated to gold particles in the conjugate pad and a 5-FU-bovine serum albumin (5-FU-BSA) The conjugate at the test line. If there is 5-FU present in the sample applied to the sample pad, it will bind to the anti-5-FU antibodies on the gold particles, leaving fewer of the antibodies available to bind to the 5-FU-BSA conjugate on the test line. Thus, increasing concentrations of 5-FU in the applied sample will result in

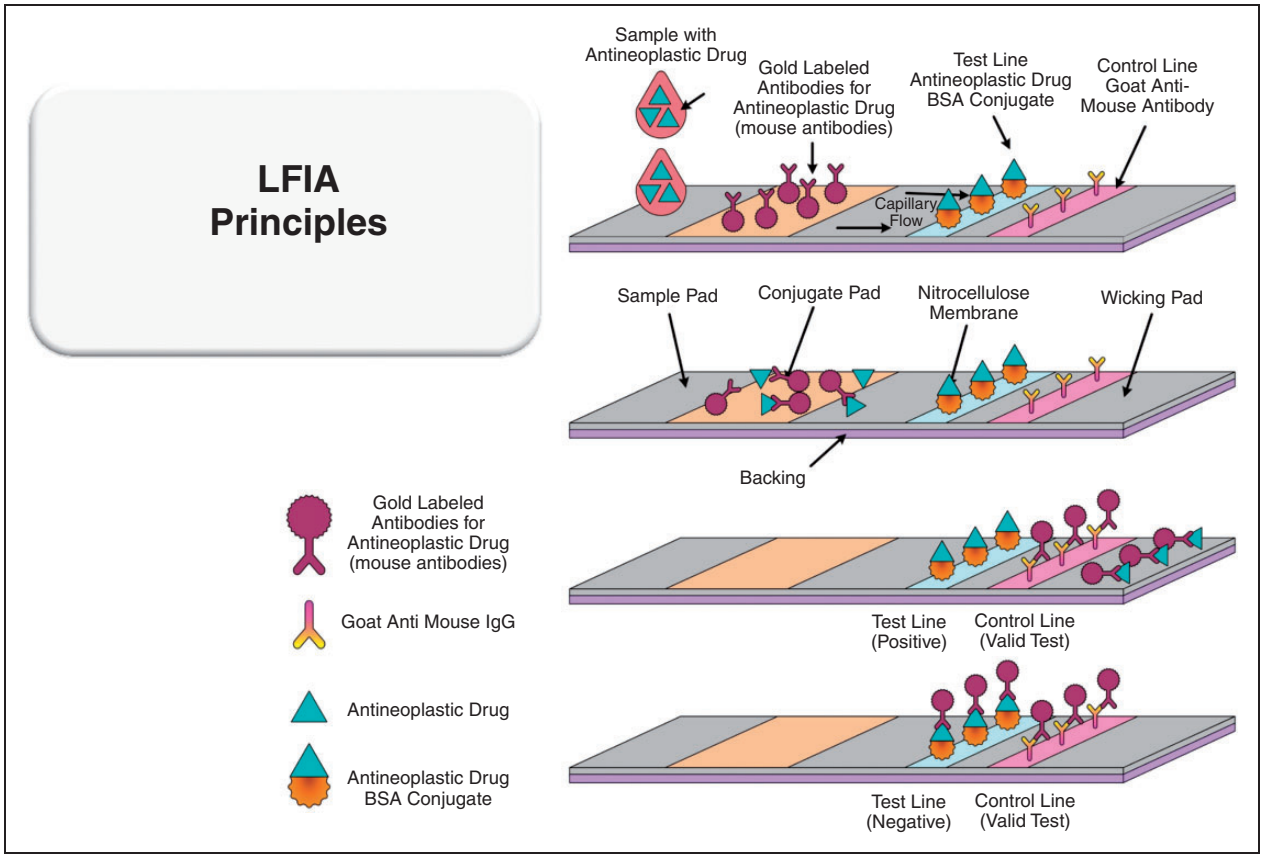

Figure 1. Schematic of Lateral Flow Immunoassay Operating Principle: Competitive assay-drug in solution binds to gold labeled antibodies resulting in less antibody binding to drug-BSA conjugate at test line. Test line becomes less intense with increasing drug concentration while control line is relatively constant.

fewer gold particles binding to the test line. Since the gold particles impart a red color to the test line, the test line will become dimmer and eventually disappear with increasing concentrations of the 5-FU in solution. There is also a control line that employs a different antibody interaction. The control should always be present and is used to indicate that the monitor is performing properly and was used with the electronic reader and visual interpretation as will be explained later.

Monitor description

The monitors are small cassettes 8 cm long, 2 cm wide by 0.4 cm thick and weigh about 4.5 g. Figure 2 shows

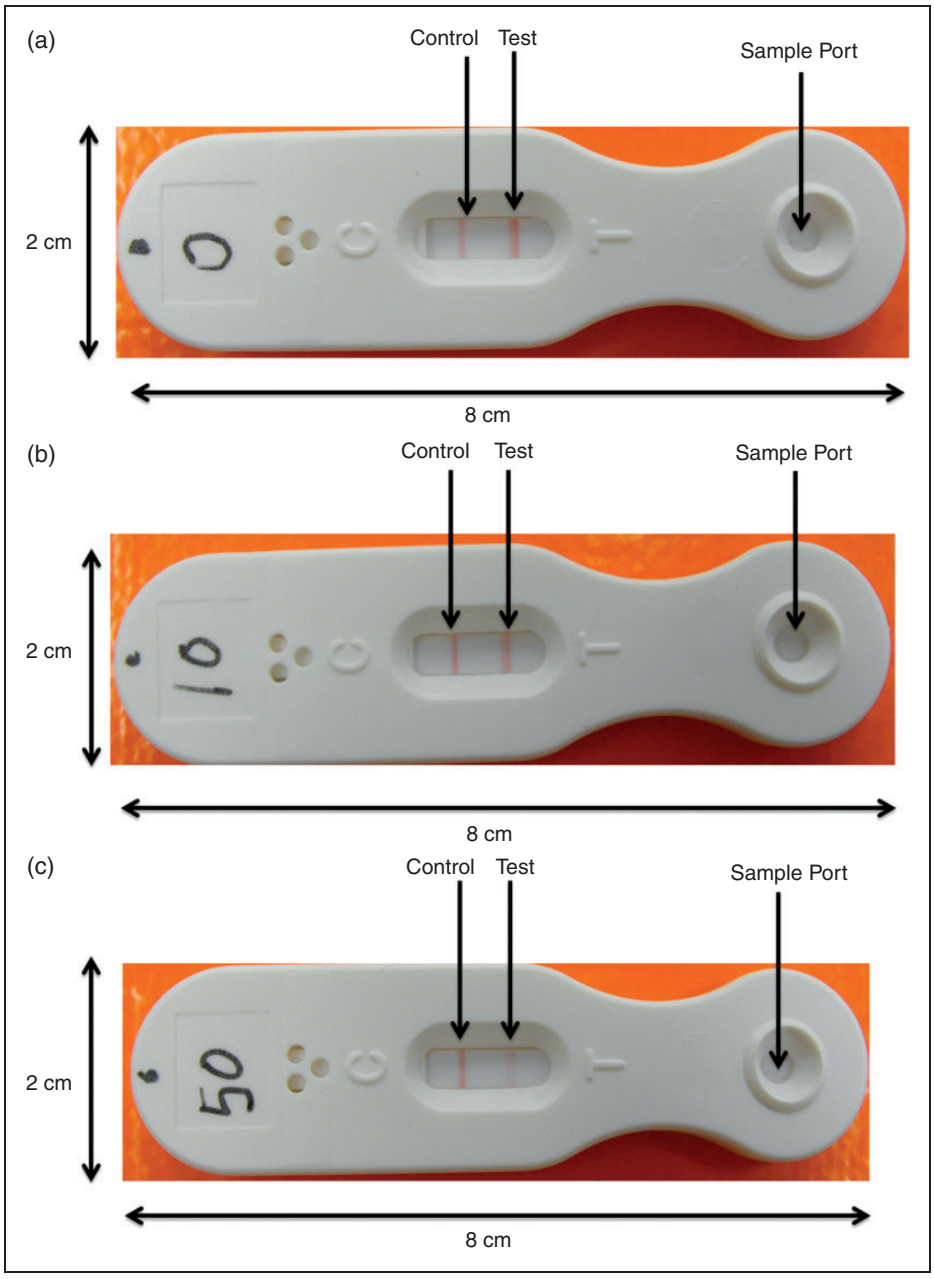

Figure 2. (a): Developed lateral flow monitor for 5-fluorouracil where the test line is more intense than control line (Ratio of control line intensity/test line intensity (C/T) = 0.56). The figure shows the test line, control line and the sample port where the sample is added. (b) Developed lateral flow monitor for 5-fluorouracil where test and control lines are about equal (C/T = 1.17). The figure shows the test line, control line, and the sample port where the sample is added. (c) Developed lateral flow monitor for 5-fluorouracil where control line is more intense than test line (C/T = 2.41). The figure shows the test line, control line, and the sample port where the sample is added.

the parts of the monitor and will be used to describe the visual interpretation procedure.

5-FU solution preparation: the stock solution of 5-FU was prepared by dissolving a weighed amount of 5-FU between 10 and 25 mg in 1 ml of concentrated ammonium hydroxide. The spiking solutions were prepared by diluting the stock solution in 10 mM ammonium acetate as required to produce desired concentrations of 5-FU. This allowed a range of 5-FU spike levels to be applied using identical spike volumes, so all tiles would dry equally before wiping.

Surface sampling

The surface sampling procedure was developed to be convenient and rapid. Ceramic bathroom tiles, vinyl floor tiles, counter top composite tiles, stainless steel tiles, and glass tiles of 100 cm^2 area were spiked with 50 µl spiking solution to give 0, 5, 10, 25, 50, or 100 ng of 5-FU surface loading (three tiles at each level) and allowed to dry for 2 h. Each 100 cm^2 surface was completely wiped with a cotton swab wetted in vial containing 1 ml of ammonium acetate-Tween 20 sampling buffer first in an up and down direction, then in a sideways direction, and finally repeating the up and down wiping directions. The swab is then returned to the vial containing sampling buffer, agitated vigorously for 2 min, and a 75 µl aliquot of the resulting extract is applied to the lateral flow drug monitor (two for each of the extracts).

Monitor testing

The monitors were tested with samples produced by wiping spiked tile surfaces with cotton swabs and extracting the swabs as described in the last section. In addition, the monitors were tested with control samples prepared (1) by directly spiking sampling buffer with spiking solution and (2) by directly spiking swabs that were then extracted. The spiked buffer controls demonstrate performance of the monitors without recovery losses or interference artifacts from the swabs or wiped surfaces. The spiked swab controls allow the contribution of the wipe media to the method performance (e.g. incomplete extraction of 5-FU from swabs) to be isolated and assessed. For spiked buffer controls, 50 µl of the appropriate spiking solution was added to 950 µl sampling buffer to yield 0, 5, 10, 25, 50, or 100 ng/ml of 5-FU. A 75 µl aliquot of the resulting extract was applied to the lateral flow drug monitor. For spiked swab controls, cotton swabs were spiked directly with 50 µl of 5-FU spiking solutions (one per spike level) and extracted with 950 µl sampling buffer using the same procedure used for the swabs from the tile wipe studies. A 75 µl aliquot of the resulting extract was applied to the lateral flow drug monitor. One tile material was tested each day, and a fresh set of 5-FU spiking solutions was made up to prepare each new set of spiked tiles, spiked buffer controls for that day, and spiked swab controls. For each tile material, three tiles were spiked at each level and two monitors were used with the solution produced from each spiked tile.

Three monitors were used with spiked sampling buffer solutions and spiked swab solutions produced for each of the five runs with different tile materials. The response of the monitors was evaluated using an electronic lateral flow reader (Hamamatsu model 10066) which read the intensity of the test and control lines. To obtain line intensities for the test and control lines with the reader, the monitor is put into a small drawer which is pushed into the reader where the measurement is made. This reader can easily be carried around since it only weighs about 1.6 kg but needs line power and a desktop or laptop PC for operation. A visual reading method was also used where the intensity of the test line was compared to the control line. This is illustrated in Figure 2 for situations where the test line is more intense than the control line, where the test line and control lines are equal, and where the control line is more intense than the test line. If the control line was more intense than the test line, then the mass was determined to be above the threshold. Thus the monitors were evaluated for semi-quantitative results using the reader and qualitative results using the visual line comparison. Since the monitors are first generation prototypes, the level of semi-quantitation is limited to identifying a concentration range for a measurement (i.e. is the measurement <10 ng/tile, >10 ng/tile<25 ng/tile, >25 ng/tile<50 ng/tile, >50 ng/tile<100 ng/tile, or >100 ng/tile, but we calculated loadings using fitted response curves for comparison with LC-MS/MS. The response for electronic and visual reading was determined 5, 10 and 15 min after adding solution to the monitors.

LC-MS/MS

The 5-FU lateral flow monitor was developed using phosphate buffered saline (PBS) with Tween 20 as sampling buffer. However, while an LC method was devised to separate 5-FU from the Tween to avoid signal suppression, this proved infeasible for the PBS; therefore, a sampling buffer using ammonium acetate with Tween 20 was used. The ammonium acetate-Tween 20 buffer gave nearly equivalent results to the PBS-Tween 20 buffer with the lateral flow monitors and was compatible with LC-MS/MS, which allowed the same solutions that were used in the lateral flow assay to be analyzed directly by LC-MS/MS. This was advantageous since it would be difficult to ensure that separate solutions for

the lateral flow assay and LC-MS/MS analysis would be equivalent. The chromatographic conditions used allowed adequate separation of 5-FU analyte from the Tween and other components. The limit of detection (LOD) was 0.3 ng/ml and the limit of quantification (LOQ) was 0.84 ng/ml with a precision of 3% or better for spiked solutions over the demonstrated calibration linear dynamic range of 1–250 ng/ml. The LC-MS/MS quantification of test samples was performed using a calibration plot from standard solutions prepared in sampling buffer at the contract laboratory Bureau Veritas North America (BVNA). LC-MS/MS analysis was done on solutions from spiked sampling buffer, spiked swabs, and spiked tiles at 0, 10, 25, and 100 ng. Only a limited number of samples could be evaluated with LC-MS/MS due to the cost of the analysis. More details about the LC-MS/MS procedure are in the Appendix.

Data interpretation

As noted earlier, the response of the monitors was evaluated with both the electronic reader and visual interpretation.

Electronic reader

The line intensities from the electronic reader were treated in two ways. %B/Bo (where B is the test line intensity at given mass and Bo is the test line intensity at 0 mass) was calculated and plotted against spiked mass of 5-FU for spiked buffer controls, spiked swab controls, and spiked tile samples. %B/Bo for all sample types was fitted against log of spiked mass to produce a calibration curve. The ratio of the Control line intensity to the Test line intensity (C/T ratio) was also calculated and plotted directly against the spiked mass to also produce a second calibration curve. The C/T ratio is easy to calculate and provides some compensation for variation in intensity of the test line for different monitors used for a given concentration since the test line and control line tend to vary in the same direction. Assessment of the ''goodness of fit'' of the %B/Bo versus log mass and C/T versus mass curves was investigated by evaluating the fit of the standards data to the %B/Bo or C/T model by 'standards recovery',[8] calculated by evaluating interpolated results from each fitted (observed) mass and comparing it to the mass of 5-FU spiked (expected mass). The recovered mass was calculated for the spiked swabs and spiked tiles by calculating the mass recovered at each calibration point using the fitted %B/Bo and C/T curves for the spiked sampling buffer controls and the %B/Bo and C/T response for the solutions from the spiked swab controls and spiked tile samples.

Visual interpretation

The visual interpretation involved evaluation of whether the Control line (C) or the Test line (T) is more intense by visually examining both lines. 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 brighter, then (C=) was used. Figure 2(a) to (c) shows monitors developed with different concentrations of 5-FU resulting in the test line being more intense than the control line, the test line and control being about equal, or the control line being more intense than the test line. The C/T ratio is given for each figure.

LC-MS/MS validation

For the LC-MS/MS validation, the masses measured via the LC-MS/MS method were correlated with the known spiked masses for spiked buffer control solutions, spiked swab controls, and tile samples. The recovered mass measured with the lateral flow monitors was also correlated with the mass measured via LC-MS/MS.

Results

Lateral flow immunoassays

Note that all data presented are for 15 min after adding the solution to the monitors; however, 5-min and 10-min data give similar results for %B/Bo (data not shown).

Spiked sampling buffer control samples. Figure 3(a) and (b) shows %B/Bo and C/T ratio as a function of ng spiked for spiked sampling buffer controls. The data shown in the figures are the average for the five sets of spiked buffer control samples, each of which was prepared and analyzed to accompany one of the tile wipe studies done on five different surfaces. Thus, each point presents the average of 15 determinations since there are five surfaces, and three monitors were used at each point of each surface. The %B/Bo shows an average% B/Bo of 67% at 5 ng 5-FU spiked with a coefficient of variation (CV) of about 10% indicating that this amount is detectable when spiked directly into the sampling buffer. The C/T ratio was fitted with a straight line as a function of spiked mass which shows good correlation. %B/Bo was fitted against the log of concentration and this fit was used to back calculate observed mass at each of the points. Likewise, the fitted C/T curve was used to back calculate observed mass. Figure 3(c) shows the observed mass as a function of expected mass using %B/Bo and C/T curves

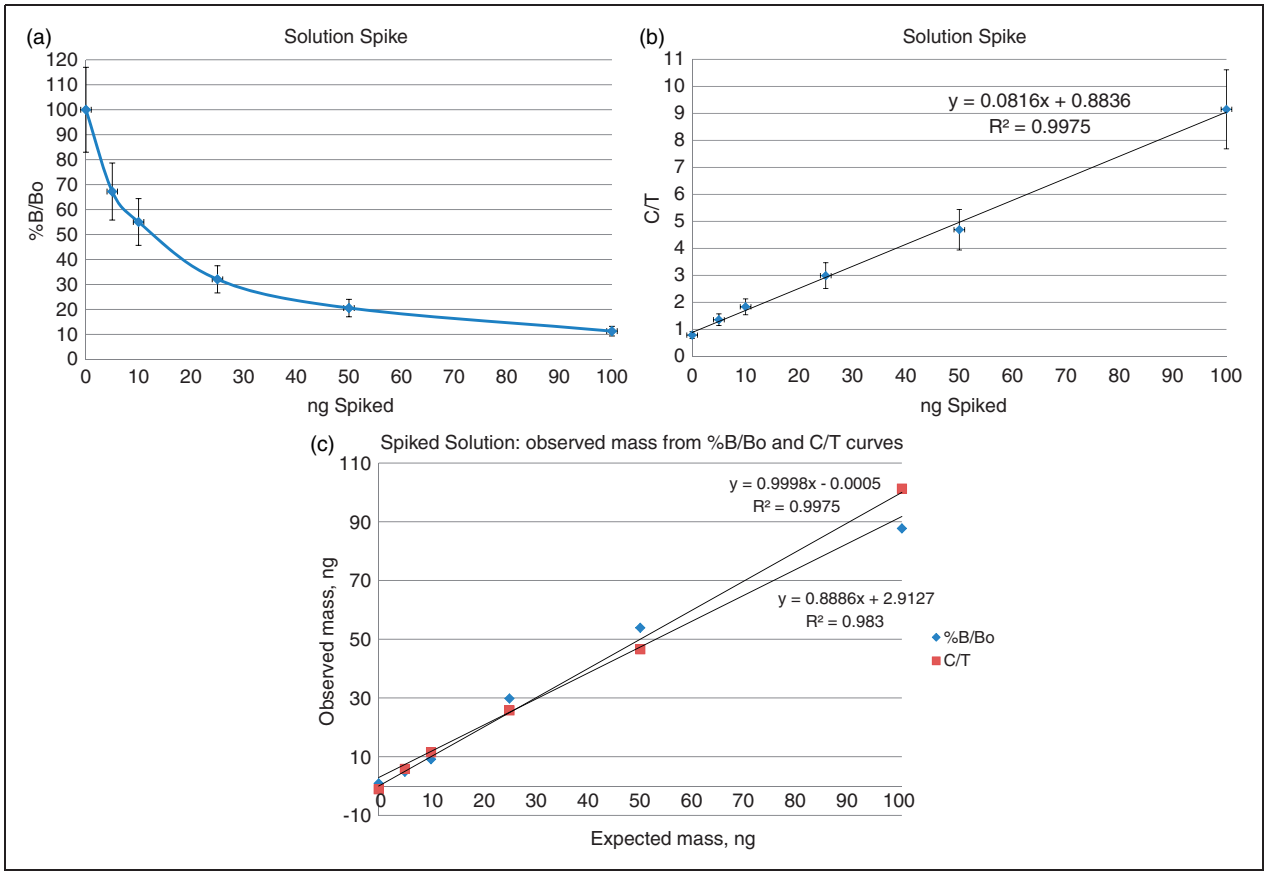

Figure 3. (a) Lateral flow monitor response for spiked sampling buffer as %B/Bo: Sampling buffer was spiked with increasing masses of 5-FU and the response of the monitors was measured and presented as %B/Bo where B is the response at a given mass and Bo is the response at 0 mass. The error bars represent the standard deviation of the measurements at each point. (b) Lateral flow monitor response for spiked sampling buffer as C/T Ratio: Sampling buffer was spiked with increasing masses of 5-FU and the response of the monitors was measured and presented as C/T ratio where C is the control line intensity and T is the test line intensity. The error bars represent the standard deviation of the measurements at each point. (c) Standards recovery for spiked sampling buffer: The standards recovery was calculated by plotting the interpolated results from either %B/Bo versus log mass or C/T versus mass fitted curves (the observed mass) against the mass of 5-FU spiked in the buffer (the expected mass).

indicating good fit for the data. The slight difference between the %B/Bo and C/T curves is not unexpected since the C/T provides some compensation for variation between individual measurements at each concentration. Table 1 shows that the visual interpretation for the spiked buffer controls and the control line (C) was judged more intense than the test line (T) at 5–10 ng spiked level.

Spiked swab control samples. Figure 4(a) and (b) shows %B/Bo and C/T ratio as a function of ng spiked for spiked swabs. The data shown are again the average for five sets of spiked swab data, each collected to accompany the tile wipe studies performed on different surfaces and each points presents the average of 15 determinations as described for the solution spikes. %B/Bo show an average of 74% with CV of 20% at 10 ng spiked indicating this mass would be detectable from spiked swabs. The C/T and %B/Bo were fitted the same way as with the solutions and used to calculate observed mass. Figure 4(c) shows the observed mass versus the expected mass as was done for the spiked buffer controls and shows good fit. As mentioned earlier, the fitted C/T and %B/Bo curves for the spiked buffer controls were used to calculate the recovered mass for spiked swab control samples and this is given in Figure 4(d) which shows good correlation but incomplete recovery of 5-FU from the swabs (plot slopes are <1.00). Table 1 shows the visual interpretation of data from spiked swabs which indicates that the control line was more intense than the test line at 10–25 ng spike level.

Spiked tile wipe samples. The data for the spiked tiles were treated in the same way as the spiked buffer controls and spiked swab controls except that the data for

Table 1. Visual interpretation of lateral flow monitor response–Most intense line (test-T, control-C) at different spiking levels.

Spiked mass (ng)	Solution	Swab	Wipes Ceramic	Wipes Vinyl	Wipes Composite	Wipes Stainless	Wipes Glass
0	T to =	T to =	T	T	T to C=	T to =	T = to C=
5	C = to C	= to C	T = to =	T = to =	= to C=	T to C=	T to =
10	C	C = to C	T = to C	T = to C	C = to C	= to C=	C = to C
25	C	C	C	C	C	C	C
50	C	C	C	C	C	C	C
100	C	C	C	C	C	C	C

Note: Monitor performance using visual interpretation: Monitors were developed with solutions from spiked sampling buffer, spiked swabs and spiked tiles. The results were evaluated by assessing visually which was the most intense line. If the test line was the most intense, then T was used, and if the control line was the most 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. There is variation in the response of individual monitors at lower levels giving a range of visual interpretations for some samples.

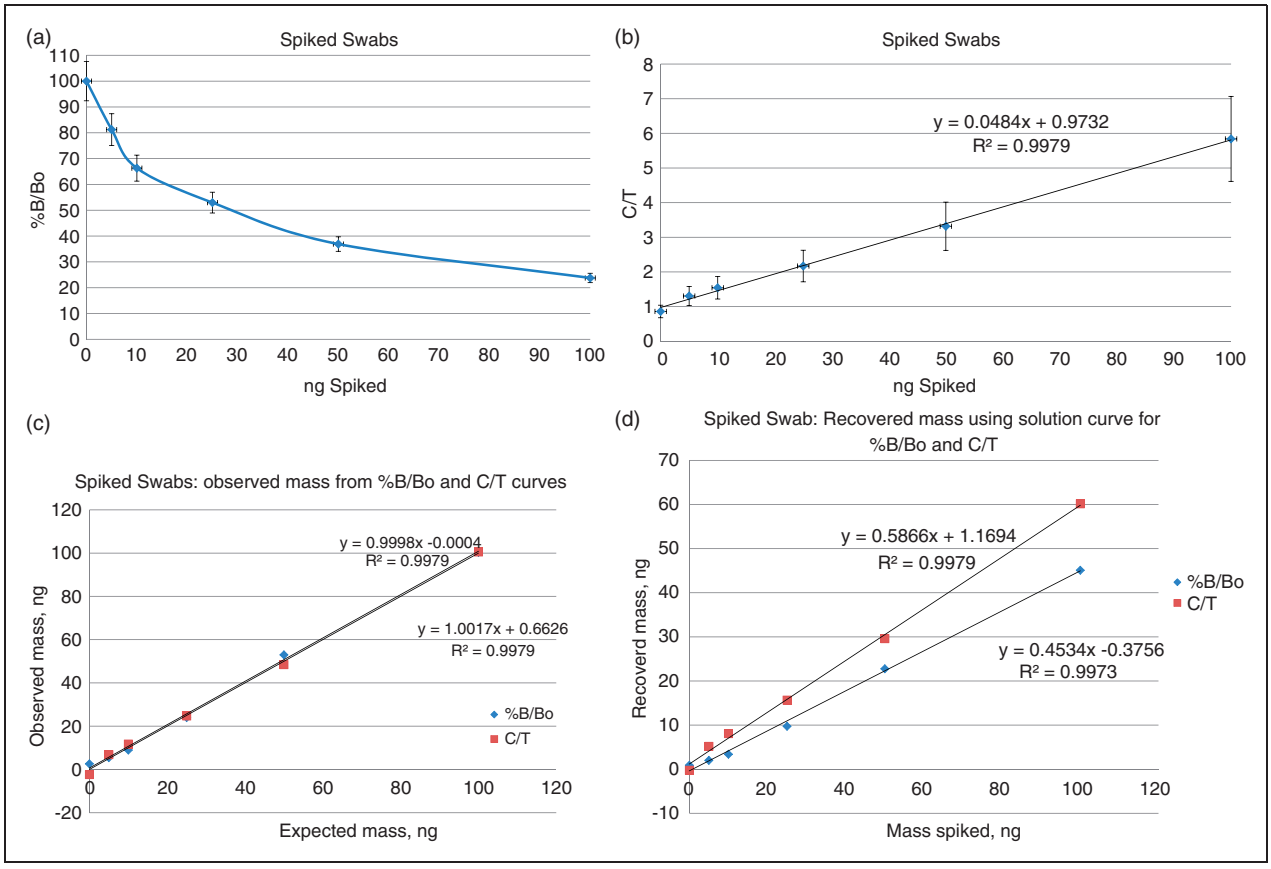

Figure 4. (a) Monitor response for solutions from spiked swabs as %B/Bo: Swabs were spiked with increasing masses of 5-FU and the response of the monitors for solutions extracted from these swabs was measured and presented as %B/Bo where B is the response at a given mass and Bo is the response at 0 mass. The error bars represent the standard deviation of the measurements at each point. (b) Monitor response for solutions from spiked swabs as C/T ratio: Swabs were spiked with increasing masses of 5-FU and the response of the monitors to solutions extracted from the swabs was measured and presented as C/T ratio where C is the control line intensity and T is the test line intensity. The error bars represent the standard deviation of the measurements at each point. (c) Standards recovery for spiked swabs: The standards recovery was calculated by plotting the interpolated results from either %B/Bo versus log mass or C/T versus mass fitted curves (the observed mass) against the mass of 5-FU spiked on the swab (the expected mass). (d) Recovery from spiked swabs using fitted solution curve: The mass recovered from the spiked swabs was calculated by taking the response of the monitors for the solutions from spiked swabs and using the fitted %B/Bo versus log mass or C/T versus mass fitted curves for spiked sampling buffer solutions to calculate the concentration.

each surface was treated separately. Each value represents an average of six lateral flow monitors since there were three tiles at each level and two lateral flow monitors for each tile. Figure 5(a) and (b) shows %B/Bo and C/T ratio as a function of ng 5-FU for spiked tile samples. The %B/Bo curves vary from surface to surface with 80% for vinyl and ceramic at 10 ng and 80% between 10 and 25 ng for other surfaces. Again, the %B/Bo data was fitted against log spiked mass and C/T was directly fitted to spiked mass, and the resulting curves were used to calculate observed mass which was correlated with expected mass as shown in Table 2 for %B/Bo and Table 3 for C/T fit. The fitted %B/Bo and C/T curve for the spiked sampling buffer controls were also used to calculate recovered mass which is shown in Table 4 for %B/Bo and Table 5 for C/T. There is good correlation but even lower recovery than with the spiked swab control samples (compare plot slopes against those in Figure 4d), as would be expected since wiping with swabs would not be expected to be 100% efficient and some surfaces may retain significant amounts of the 5-FU spike (below). Table 1 shows the visual interpretation for different surfaces which shows that all surfaces have the control line more intense than the test line at 25 ng spike levels.

LC-MS/MS. The LC-MS/MS measurements were done on spiked sampling buffer controls, extracts from spiked swab controls and spiked tiles, using the remaining solutions which were sent to the contract lab after the lateral flow monitor analyses were complete. Table 6 shows the correlation of the recovered mass measured from LC-MS/MS with the spiked mass for each type of sample. As noted earlier, these recoveries are determined using an independent calibration curve prepared by the LC-MS/MS analyst using new standards prepared in sampling buffer while the lateral flow monitors used the spiked sampling buffer control sample data for calibration. For the surface wipe samples, we report both an overall correlation and a correlation for each surface type since the surfaces gave different recoveries. Table 7 gives the correlation of the recovered mass from the lateral flow monitors versus recovered mass via LC-MS/MS. The recovered mass for the lateral flow monitors used the C/T values from each surface sample type determined with the fitted spiked buffer control curve generated on the same day. For the wipe samples, the correlation was done for (1) all the data including 100 ng spiked and (2) for spiked samples less than 25 ng since the absolute values of those data (slope of plot being closer to 1) agreed better with the LC-MS/MS results. 100 ng is the only spike level above 25 ng for which a value was determined via LC-MS/MS.

Discussion

The good correlation (slope = 1.01, intercept = −0.23, $R^2 = 0.9999$) of the LC-MS/MS measurement with 5-FU mass for spiked buffer controls indicates that the spiking technique used to prepare in-house test samples for this investigation was accurate (Table 6). It also indicates that although the calibration standards prepared for LC-MS/MS analysis used a different 5-FU supply than in-house samples, quantitative accuracy of prepared samples was reliable.

For spiked sampling buffer controls, 5 ng of 5-FU can be detected using the lateral flow monitors since this %B/Bo was an average of 67% at this spiked mass. The response curve can be modeled with %B/Bo versus log spiked mass or C/T versus spiked mass and both give good correlation of observed versus expected mass (Figure 3c). The C/T is easy to calculate and gives some compensation for variation in the lateral flow monitors since the test line and control line intensity tend to rise and fall together. The visual interpretation indicates that 5 ng spiked mass is detectable since all monitors give C = to C at this mass and all are C for the 10 ng spike level.

For the spiked swab controls, the average %B/Bo was 74% at 10 ng indicating that this mass of 5-FU can be detected. The response could be modeled with %B/Bo versus log mass or C/T versus mass and the observed versus expected mass gave good correlation with either fit (Figure 4c). However, the calculated recovery obtained using the spiked buffer control sample curve gave about 50% recovery for both the %B/Bo and C/T curves (see respective slope values, Figure 4d). The LC-MS/MS results also indicated incomplete recovery from the spiked swabs with an average of 86% which agrees well with the slope when the recovery is plotted against mass (Table 6). However, the LC-MS/MS recovery values are higher than those from the lateral flow monitor as indicated by slope value <1.00 for swab samples in Table 7 which correlates the two methods. Visual interpretation indicated that 10 ng 5-FU is detectable since all monitors showed that C = to C by that mass.

For the spiked tiles, %B/Bo curves varied from surface to surface (Figure 5a) and indicated that 10 ng of 5-FU could be detected on ceramic and vinyl tiles and 10–25 ng on other surfaces. There is good correlation of observed mass with expected mass using the fitted curves for both %B/Bo and C/T for all the surfaces (Tables 2 and 3). Calculation of the recovered mass using the spiked buffer control curve indicates generally good correlation but incomplete recovery (see respectively R^2 and slope values in Tables 4 and 5) as would be expected from limitations in the wiping technique. LC-MS/MS also indicates incomplete recoveries from tile wipe samples (indicated by slope values, Table 6).

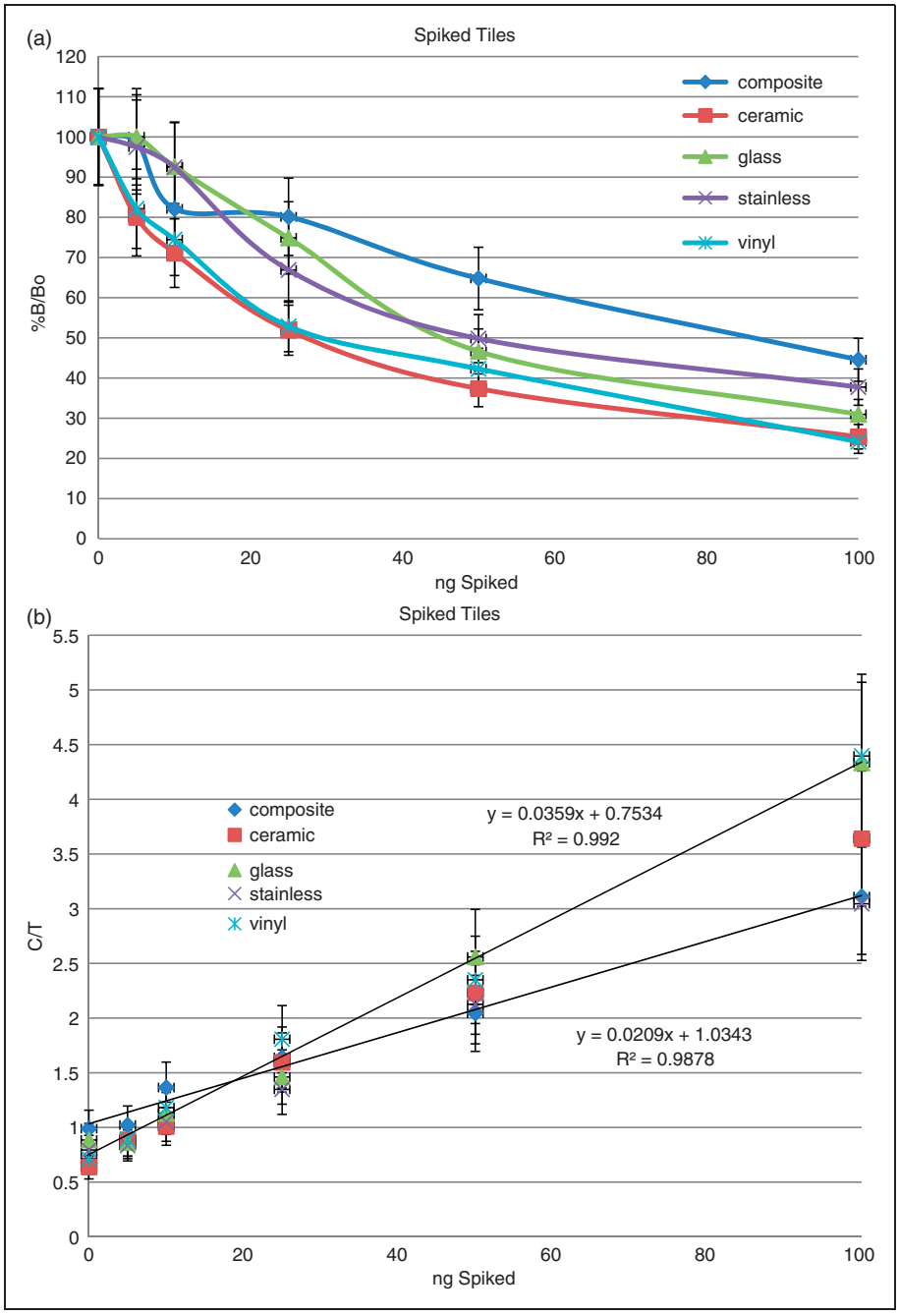

Figure 5. (a) Monitor response for solutions from spiked tiles as %B/Bo: Tiles of 100 cm^2 area and various surfaces were spiked with increasing masses of 5-FU and the response of the monitors for solutions extracted from swabs used to wipe these tiles was measured and presented as %B/Bo where B is the response at a given mass and Bo is the response at 0 mass. The error bars represent the standard deviation of the measurements at each point. (b) Monitor response for solutions from spiked tiles as C/T ratio: Tiles of 100 cm^2 area and various surfaces were spiked with increasing masses of 5-FU and the response of the monitors for solutions extracted from swabs used to wipe these tiles was measured and presented as C/T ratio where C is the control line intensity and T is the test line intensity. Regression lines for all surface types are shown; however, only the regression equations and error bars of the vinyl and the composite are shown on the graph. The regression for the vinyl yielded the highest slope and that for the composite yielded the lowest slope. The slopes for all other surfaces (not shown) are in between vinyl and composite. The error bars represent the standard deviation of the measurements at each point.

Table 2. Observed mass versus expected mass for fitted %B/Bo versus log mass calibration curve.

	Slope	Intercept	R^2
Ceramic	1.0001	0.5636	0.9983
Composite	1.3292	−5.4721	0.9357
Glass	1.0989	−0.1767	0.9835
Stainless	1.0252	1.1418	0.9874
Vinyl	1.0922	−1.4521	0.9866

Note: Observed mass versus expected mass for Fitted %B/Bo versus Log Mass Calibration Curve for Spiked Tiles: Tiles were spiked with increasing masses of 5-FU, wiped, and the solution was applied to the lateral flow monitors. The response of the monitors was fitted as %B/Bo as a function of log mass and this relationship was used to calculate the observed mass which was plotted against the expected mass.

Table 3. Observed mass versus expected mass for fitted C/T versus mass calibration curve.

	Slope	Intercept	R^2
Ceramic	0.7467	0.0002	0.9957
Composite	0.9986	−0.0002	0.9878
Glass	1.0012	−0.0002	0.9939
Stainless	0.9986	−0.0002	0.9867
Vinyl	0.9993	0.0012	0.992

Note: Observed Mass versus Expected Mass for Fitted C/T versus Mass Calibration Curve for Spiked Tiles: Tiles were spiked with increasing masses of 5-FU, wiped and the solution was applied to the lateral flow monitors. The response of the monitors was fitted as C/T (the ratio of the control line intensity to the test line intensity) as a function of mass, and this relationship was used to calculate the observed mass which was plotted against the expected mass.

There is good correlation of the recovery values from LC-MS/MS with those calculated from the lateral flow assay (R^2 values in Table 7), although absolute values of calculated recoveries from the immunoassay are lower (slope values in Table 7 are <1.00). The absolute values agree better for mass values 25 ng or less. The exception is stainless steel wipe samples for which there is little difference in agreement. Insufficient LC-MS/MS data were available to allow ranges to be compared for glass wipe samples.

Variable 5-FU recoveries from different surfaces have been shown in another study using LC-MS/MS (Pretty et al, 2010). The surfaces were selected to represent a wide range of characteristics for testing purposes, and surface roughness and porosity are expected to affect wiping efficiency. However, in this study, the relative efficiencies on surfaces are different from those reported in the previous LC-MS/MS study, which may be due to the different surface sampling techniques employed. Parameters such as sampling buffer pH and additive content can affect wiping efficiency as

Table 4. Recovered mass using fitted solution %B/Bo versus log mass calibration curve.

	Slope	Intercept	R^2
Ceramic	0.34	0.6781	0.9977
Composite	0.1321	0.082	0.957
Glass	0.4216	−2.2471	0.9731
Stainless	0.2721	−0.1914	0.9874
Vinyl	0.37	0.1702	0.9913

Note: Recovered mass from the spiked tiles using Fitted Spiked Sampling Buffer Solution %B/Bo versus Log Mass Calibration Curve: Tiles were spiked with increasing masses of 5-FU, wiped, and the solution was applied to the lateral flow monitors. The fitted %B/Bo versus log mass relationship for the spiked sampling buffer was used with monitor response from spiked tiles to calculate the recovered mass which was plotted against spiked mass.

Table 5. Recovered mass using fitted solution C/T versus mass calibration curve.

	Slope	Intercept	R^2
Ceramic	0.254	0.0621	0.9957
Composite	0.2324	2.8708	0.9878
Glass	0.7426	−6.571	0.9939
Stainless	0.3343	−1.8595	0.9867
Vinyl	0.4008	−1.2632	0.992

Note: Recovered mass from the spiked tiles using Fitted Spiked Sampling Buffer Solution C/T versus Mass Calibration Curve: Tiles were spiked with increasing masses of 5-FU, wiped, and the solution was applied to the lateral flow monitors. The fitted C/T versus mass relationship for the spiked sampling buffer was used with monitor response from spiked tiles to calculate the recovered mass which was plotted against spiked mass.

Table 6. Correlation of LC-MS/MS measured mass and spiked mass.

	Slope	Intercept	R^2
Solution	1.0185	−0.2358	0.9999
Swab	0.8276	0.5247	0.9998
Wipes overall	0.5039	−0.5905	0.8968
Wipes ceramic	0.4116	−0.4191	0.9984
Wipes composite	0.4759	−0.574	0.9994
Wipes glass	0.3882	0.0418	0.9998
Wipes stainless	0.3887	0.6229	0.9944
Wipes vinyl	0.7368	−1.3006	0.998

Note: Correlation of LC-MS/MS measured mass and spiked mass: The mass measured by the LC-MS/MS was correlated with spiked mass for spiked sampling buffer solutions, spiked swab solutions and spiked tile solutions (overall and by tile type).

Table 7. Correlation of lateral flow monitor recovered mass with LC-MS/MS recovered mass.

	Slope	Intercept	R^2
Solution	0.9923	0.5107	0.9984
Swab	0.7341	0.6572	0.9985
Wipes overall 0–100	0.5894	0.3374	0.9267
Wipes overall 0–25	0.8192	−1.0296	0.8426
Wipes ceramic 0–100	0.6095	0.4502	0.9899
Wipes ceramic 0–25	0.9017	−0.7058	0.983
Wipes composite 0–100	0.4365	3.1433	0.9953
Wipes composite 0–25	0.5233	2.7555	0.9505
Wipes glass 0–25	1.0748	−3.6148	0.9123
Wipes stainless 0–100	0.9218	−2.418	0.9999
Wipes stainless 0–25	0.9218	−2.418	0.9995
Wipes vinyl 0–100	0.5469	0.0382	0.9913
Wipes vinyl 0–25	0.8059	−1.6581	0.9888

Note: Correlation of lateral flow monitor recovered mass with LC-MS/MS recovered mass: The calculated recovered mass measured with the lateral flow monitors was plotted against the recovered mass measured with the LC-MS\MS for spiked sampling buffer solutions, spiked swab solutions, and spiked tile solutions (overall and by tile type with mass ranges 0–100 and 0–25 ng).

well as the type of swab media used. The cotton swabs used for wipe sampling are manufactured in bulk without a high degree of quality control, which can affect performance. Two different brands of the swabs were shown to have different recoveries as shown by analysis of spiked swab extracts via LC-MS/MS. In addition, one lot of swabs from one of the brands was completely incompatible with the lateral flow assay. These observations show that each batch of swabs should be assessed for suitable performance with the lateral flow monitors prior to field study work, since reliable performance cannot be guaranteed in advance.

These results show that when the monitors are used with a sampling technique using the cotton swabs, 25 ng 5-FU can be detected visually and less than 25 ng can be detected using the electronic reader. There are probably surface sampling techniques that could give greater recovery. The cotton swabs themselves have less than 100% recovery for extraction of 5-FU and the wiping technique using the cotton swabs is also less than 100% efficient. Some surfaces may not release all 5-FU that is present to the swab; therefore, different surfaces may yield variable sensitivity. However, the present technique is simple and easy to use. Other sampling techniques might also require dilution of the sample in greater volumes of sampling buffer which would mean that they would actually be less sensitive in terms of mass detected on the surface.

Field studies are being conducted to assess the performance of the monitors in healthcare settings. In these studies, the response of the monitors is being compared to LC-MS measurements on the same solutions that are used with the monitors. This will allow the effects of possible interferences to be assessed and the comparison of the concentration measurements by the two methods. If the measured concentrations are above the monitors' upper limit, which is 100 ng/100 cm^2, then the sample can be diluted to bring the concentration within the measurement range of the monitors. However, in practice, the sampled area would likely be decontaminated and then reassessed for remaining contamination since the goal of the monitors is to detect and remove contamination to help lower worker exposure. The level at which contamination needs to be removed will have to be determined by users since there is no set limits for contamination. The monitors could also be used to evaluate work practices to help lower contamination. The level of contamination can be measured immediately after a process such as drug preparation or administration is performed. The procedure could then be altered to see if the alternate procedure lowered the level of contamination. The improved procedure would be difficult to develop and evaluate without near real time assessment of contamination. The monitors have the advantage that they are simple to use so workers themselves can evaluate contamination and assess work practices. Other analytical techniques require taking the sample to a laboratory where a trained analyst performs the analysis. In order to be used widely, the monitors will have to be available from a commercial source. A healthcare company has partnered with NIOSH for the field testing and further development of the 5-FU monitor and monitors for other drugs. The goal is to commercially produce monitors that can give near real time measurement of contamination at a cost that will allow frequent assessment of contamination.

Conclusion

These laboratory studies indicate that the 5-FU detection technique employing surface wiping and lateral flow monitors provides a sensitivity of 25 ng/100 cm^2 (0.25 ng/cm^2) or better in a combined sampling and analysis time of 15 min or less. The technique is

simple, uses equipment that is easily transported to a worksite and could be employed by personnel with limited training. Note that this study used a lateral flow reader that is fairly easy to transport and operate in remote sites, but does require line power and an external PC for operation. However, there are many readers available for clinical applications that are portable and self-contained so that they can perform calculations and store data. Some of these readers use cell phone cameras to evaluate line intensities and can report results to cloud databases. The monitor is easy to use and provides a rapid determination of surface contamination, which will enable healthcare workers to evaluate work sites for antineoplastic drugs, thus reducing their exposure to these hazardous drugs. The assay can be used to identify processes producing contamination and verify that clean-up processes are effective. Field studies are needed to assess the utility of the technique in actual healthcare workplace settings. These field studies will be compared to literature from past studies concerning levels of contamination measured. As noted earlier, these monitors are first generation prototypes and further development can produce improved monitors that will be more reproducible. The monitor is limited to the measurement of 5-FU, but monitors for other antineoplastic drugs are also being developed to include a wider range of drugs. The LC-MS/MS technique will be useful in assessing the performance of the lateral flow monitors in the field.

Disclaimer

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Appendix 1

The LC-MS/MS method was developed to measure 5-FU directly in the ammonium acetate-Tween® 20 sampling buffer used for the lateral flow monitors. The gradient chromatographic conditions used allowed adequate separation of 5-FU analyte from the Tween along with other components and ensured that the Tween did not build up on the column through repeated injections of sample, which was observed with an earlier isocratic method. The HPLC column used was YMC-ODS-AQ, 2.0 × 250 mm, 5 µm (Waters Part No. AQ12S052502WT, Waters Corp, Milford, MA, USA) at a column temperature of 30°C. The mobile phase flow rate was 0.28 ml/min with mobile phase components A and B (A – 2 mM aqueous ammonium acetate, B – Methanol). A gradient of these components was used as follows:

Isocratic 95% A, 5% B	0–4.5 min
Gradient to 5% A, 95% B	4.5–5.5 min, linear ramp
Isocratic 5% A, 95% B	5.5–11 min
Gradient to 95% A, 5% B	11–12 min, linear ramp
Isocratic 95%A, 5% B (re-equilibration)	12–17 min

A 15 µL sample volume was injected onto the column and the retention time of 5-FU was approximately 4.5 min with a total run time of 17 min including re-

equilibration. The triple quadrupole mass spectrometer (Micromass Quattro LC (Waters Corp)) was operated in electrospray negative ion ionization mode using multiple reaction monitoring for the following transitions:

5-fluorouracil (analyte): mass to charge (m/z) 129 to 42
5-fluorouracil-$^{15}N_2$ (Internal standard at 50 ng/ml level): m/z 131 to 43

The transitions were measured with a dwell time of 200 ms for each transition. Electrospray capillary voltage was 500 V, cone voltage was 30 V, and fragmentation collision energy was 14 eV for both transitions. The validated linear calibration range was 1–250 ng/ml 5-FU in sampling buffer which allowed all test samples to be analyzed without prior dilution. Limits of detection and quantification evaluated using a regression plot generated from low level 5-FU standards prepared in sampling buffer were 0.3 and 0.84 ng/ml, respectively. This is also equivalent to 0.3 and 0.84 ng 5-FU in 1 ml test samples.