



Detection and measurement of surface contamination by multiple antineoplastic drugs using multiplex bead assay

Jerome P Smith, Deborah L Sammons, Shirley A Robertson, Jack R Pretty, D Gayle DeBord, Thomas H Connor and John E Snawder

Abstract

Objectives: Contamination of workplace surfaces by antineoplastic drugs presents an exposure risk for healthcare workers. Traditional instrumental methods to detect contamination such as liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) are sensitive and accurate but expensive. Since immunochemical methods may be cheaper and faster than instrumental methods, we wanted to explore their use for routine drug residue detection for preventing worker exposure.

Methods: In this study we examined the feasibility of using fluorescence covalent microbead immunosorbent assay (FCMIA) for simultaneous detection and semi-quantitative measurement of three antineoplastic drugs (5-fluorouracil, paclitaxel, and doxorubicin). The concentration ranges for the assay were 0–1000 ng/ml for 5-fluorouracil, 0–100 ng/ml for paclitaxel, and 0–2 ng/ml for doxorubicin. The surface sampling technique involved wiping a loaded surface with a swab wetted with wash buffer, extracting the swab in storage/blocking buffer, and measuring drugs in the extract using FCMIA.

Results: There was no significant cross-reactivity between these drugs at the ranges studied indicated by a lack of response in the assay to cross analytes. The limit of detection (LOD) for 5-fluorouracil on the surface studied was 0.93 ng/cm² with a limit of quantitation (LOQ) of 2.8 ng/cm², the LOD for paclitaxel was 0.57 ng/cm² with an LOQ of 2.06 ng/cm², and the LOD for doxorubicin was 0.0036 ng/cm² with an LOQ of 0.013 ng/cm².

Conclusion: The use of FCMIA with a simple sampling technique has potential for low cost simultaneous detection and semi-quantitative measurement of surface contamination from multiple antineoplastic drugs.

Keywords

Antineoplastic drugs (5-fluorouracil, paclitaxel, doxorubicin), surface analysis, multiplex measurement

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 safer handling procedures.^{4,5} National Institute for Occupational Safety and Health (NIOSH) has developed an alert where information

concerning the effects of exposure are given, processes producing exposure are described, and procedures for

Division of Applied Research & Technology, National Institute for Occupational Safety & Health, Cincinnati, OH, USA

Corresponding author:

Jerome P Smith, Division of Applied Research and Technology, National Institute for Occupational Safety and Health, US Centers for Disease Control and Prevention, 4676 Columbia Parkway, Cincinnati, OH 45226, USA.
Email: jps3@cdc.gov

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} Commercial preparations used in administering drugs to patients have concentrations of 50 mg/ml for 5-fluorouracil, 6 mg/ml for paclitaxel, and 2 mg/ml for doxorubicin, so spillage of a low volume of the preparations can result in significant levels of contamination.

Analytical techniques for measurement of surface contamination by antineoplastic drugs such as liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS)⁶ are sensitive, specific, and accurate, but the initial equipment investment is expensive and a trained analyst is required to operate the instrument so these methods often cannot be used on a frequent basis.

A technique called fluorescence covalent microbead immunosorbent assay (FCMIA) for performing multiple immunochemical determinations simultaneously has been developed by Luminex Corporation.^{7,8} FCMIA combines several classical methodologies: immunoassays, microspheres, and flow cytometry technology. In FCMIA, immunoassays are performed on sets of solid support microspheres with different characteristic internal fluorophores that allow multiple assays to be performed simultaneously (multiplexing). FCMIA has predominantly been used for multiple protein and nucleic acid analytes such as multiple antibodies in serum,⁹ multiple cytokines in serum,¹⁰ and multiple RNA and DNA viruses in patient samples.¹¹ FCMIA assays are simple to set up and the instrument does not require an expert to operate.

Previously we developed an assay and sampling technique, capable of evaluating multiple drugs of abuse on surfaces using FCMIA,¹² which has been used for exposure assessments in law enforcement evidence vaults (NIOSH HHEs 2012-0083-3189 and 2010-0017-3133). We have also developed an easily performed technique to evaluate surface contamination by antineoplastic drugs in near real time (not yet published). Healthcare workers themselves, without the need of an analytical chemistry expert, can determine results within 15 min of sampling. In this report the feasibility of an FCMIA technique for surface sampling and detection of multiple antineoplastic drugs is examined. Since there are no workplace standards for surface contamination by antineoplastic drugs, we aimed for detection of less than 1 ng/cm² and measurement of less than 5 ng/cm² of multiple antineoplastic drugs with a convenient and simple sampling technique. These levels will be useful in assessing contamination to control exposure. 5-Fluorouracil, paclitaxel, and doxorubicin were chosen for study because these are commonly used drugs and reagents for FCMIA are available.

Methods and materials

Reagents

Paclitaxel and doxorubicin standards were prepared by dissolving a weighed amount (about 1 mg) of solid Paclitaxel (Sigma T7402, Sigma Aldrich, St Louis, MO) or solid doxorubicin (Sigma 44583-1 mg) in 1 ml methanol. The 5-fluorouracil standard was prepared by dissolving a weighed amount of 5-fluorouracil (Sigma F6627-1G) in concentrated ammonium hydroxide (product A669-500, Fisher Scientific, Fair Lawn, NJ). Fresh 5-fluorouracil standard was prepared before each experiment.

5-Fluorouracil–bovine serum albumin (BSA), doxorubicin–BSA, and paclitaxel–BSA conjugates were purchased from Saladax Biomedical (Bethlehem, PA). These were produced by binding the drugs or drug analogs to the carrier protein (BSA). These conjugates are then tested for specific binding of anti-drug antibodies by the manufacturer. Monoclonal antibodies to 5-fluorouracil, paclitaxel, and doxorubicin were obtained from Saladax or Lampire Biological Laboratories (Pipersville, PA). Microspheres were obtained from Luminex Corporation (Austin, TX). Activation buffer (0.1 M NaH₂PO₄, pH 6.2), coupling buffer (0.05 M 2-(N-morpholino) ethanesulfonic acid (MES), pH 5.0), wash buffer (phosphate buffered saline (PBS), 138 mM NaCl, 2.7 mM KCl, containing 0.05% Tween[®] 20), and storage/blocking buffer (PBS, 1% BSA, 0.05% NaN₃, pH 7.4) were supplied or prepared with reagents supplied by Sigma Chemical Co. Biotin labeled anti-mouse IgG, 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (EDC), and *N*-hydroxysulfosuccinimide, sodium salt (sulfo-NHS) were obtained from Pierce Biotechnology, Inc. (Rockford, IL). Streptavidin R–phycoerythrin (streptavidin R–PE) was obtained from Molecular Probes (Eugene, OR).

Details concerning the multiplexed assay are given in a previous publication,¹² so the assay will be briefly summarized here.

Microsphere preparation

Microspheres were 5.6 μm in diameter with surface carboxylate groups for covalent attachment of biomolecules. Internally, the microspheres were dyed with red and infrared emitting fluorochromes. By proportioning the concentrations of each fluorochrome, spectrally addressable microsphere sets were obtained. The carboxylate groups on the microspheres were activated with a mixture of EDC and NHS in activation buffer and the three drug–BSA conjugates in coupling buffer were coupled to separate unique sets of carboxylate-modified (Luminex) microspheres.

Multiplexed assay

The assay is a competitive assay, where drug in solution competes with a microsphere bound drug-BSA conjugate for an anti-drug antibody. This results in less anti-drug antibody being bound to the microsphere at higher drug concentrations. The anti-drug antibody bound to the microsphere is detected with a labeled secondary antibody (biotin labeled anti-mouse IgG) which in turn binds a fluorescent label (streptavidin R-PE). Thus the streptavidin R-PE fluorescent signal from the microsphere decreases with increasing drug concentration. The assay was performed in a 1.2 μ m filter membrane microtiterplate (Millipore Corp., Part #MABVN1250, Bedford, MA) which allowed wash steps between the addition and incubation of primary antibody, secondary antibody, and streptavidin R-PE fluorescent label. After the steps of the assay were complete, the microtiterplate was placed in the autosampler platform of the LUMINEX 100 (Luminex) instrument for reading using software, calibration microspheres, and sheath fluid supplied by the manufacturer. The instrument was programmed to collect data from 100 microspheres for each analyte (classified by their internal fluorescence ratio) and acquire the median fluorescence intensity (MFI) of the microsphere-drug-BSA conjugate-primary anti-drug IgG antibody-secondary-anti-IgG-biotin-streptavidin R-PE complex. The analysis of a sample containing 5-fluorouracil, paclitaxel, and doxorubicin can be accomplished in less than 60 s using this system.

Mixtures of the three drugs were prepared in eight concentrations shown in Table 1 in storage/blocking buffer to measure the response of the assay. The range of concentrations of the standard solutions for each assay was determined by the known response of the assays.

Table 1. Concentration of drugs in standard solutions.^a

Standard solution	5-Fluorouracil (ng/ml)	Paclitaxel (ng/ml)	Doxorubicin (ng/ml)
1	1000	100	2
2	500	50	1
3	250	25	0.5
4	125	12.5	0.25
5	62.5	6.25	0.125
6	31.25	3.125	0.0625
7	15.6	1.5625	0.0312
8	0	0	0

^aThe concentration of 5-fluorouracil, paclitaxel, and doxorubicin in each standard solution used to determine the response of the assay.

Data analyses

Standard curves were constructed from four-parameter logistic-log fits (4-PL, SigmaPlot, SPSS, Chicago, IL) of % B/B_0 data (where B = the MFI for each individual drug standard and B_0 = the MFI measured for the corresponding blank) versus ng/ml of standard. The limit of detection (LOD) of the assays was defined as 90% B/B_0 and was interpolated mathematically from the coefficients of the 4-PL equations. 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”,¹³ calculated by evaluating interpolated results from each 4-PL fit (observed concentration) and comparing them to the concentrations of drugs added to the system (the expected concentration) using the following relationship:

$$\begin{aligned} \% \text{ recovery} \\ = 100 \times (\text{observed concentration from 4 - PL} \\ \text{fit of data/expected concentration}) \end{aligned}$$

The resultant data were analyzed for linearity from the FCMIA LOD of each drug to highest level studied by linear regression with the slope indicating recovery and the correlation coefficient giving goodness of fit.

To test for cross-reactivity among the drugs, increasing concentrations of the individual drugs were added to the mixture of 5-fluorouracil-BSA, paclitaxel-BSA, and doxorubicin-BSA microspheres and anti-5-fluorouracil, anti-paclitaxel, anti-doxorubicin antibodies, and the assays were run as above. The cross-reactivity was determined by the response of each assay to analytes other than its own.

Recovery of drugs from surfaces

To study the recovery of drugs from surfaces, 10 cm x 10 cm square ceramic tiles (100 cm² area) with glazed nonporous surfaces were spiked with solutions of the drugs in methanol and the solution was allowed to dry to produce the desired surface loadings. The concentrations of the spiking solutions were adjusted so that spiking the tiles with 50 μ l would produce the desired surface loading. Three tiles were spiked at each level. The tiles were spiked with more than the individual assays' upper limit which was adjusted according to the measured sampling efficiency. The surface loadings produced for the different drugs are shown in Table 2. The surface was then sampled using a swab (product no 71-7020, Super Brush LLC, Springfield, MA) wetted in wash buffer. The sampling was done by carefully wiping the surface with the wetted swab in one

Table 2. Mass of drugs on tiles.^a

Tile	5-Fluorouracil (ng/tile)	Paclitaxel (ng/tile)	Doxorubicin (ng/tile)
1	2000	1000	5
2	1000	500	2.5
3	500	250	1.25
4	250	125	0.625
5	125	62.5	0.312
6	62.5	31.25	0.156
7	31.25	15.625	0.0781
8	0	0	0

^aThe mass of 5-fluorouracil, paclitaxel, and doxorubicin on the tiles used to determine the recovery of the drugs from the surface.

direction with an overlapping pattern, then repeating the same wiping pattern in a direction perpendicular to the first direction, and finally repeating the original wiping pattern. The swab was then placed in a glass vial containing 1 ml of storage/blocking buffer and the swab was extracted with vigorous shaking for 2 min. The resulting solution was run in the assay without any further dilution. The recovery was determined by comparing the concentration of the solution from wiped tile with the concentration calculated from the spiked masses of the drugs assuming 100% recovery. The recovered mass was fitted to the spiked mass using least squares fit. The recovery from the surface was evaluated by the slope of the recovered mass curve. The LOD was calculated as the spike mass equivalent to the blank (tile spiked with 0) + 3 times the standard deviation of the blank and the lower limit of quantitation (LOQ) was calculated as the spiked mass equivalent to the blank + 10 times the standard deviation of the blank using the recovered mass versus spiked mass relationship.

Results

Response curves

Note that all data for the response curves are for the three drug analytes being measured simultaneously. The data shown are the average of six different runs for 5-fluorouracil and paclitaxel and three runs for the doxorubicin with the standard deviation shown as error bars. There were fewer runs for the doxorubicin because the range had to be changed since it is the most recently developed assay and was found to be not linear for the more extended range originally used. Due to the difference in the range for the assays, the following concentration ranges were used for standard curves: 5-fluorouracil 0–1000 ng/ml, paclitaxel 0–100 ng/ml, and doxorubicin 0–2 ng/ml.

5-Fluorouracil

Figure 1a shows % B/B_0 for 5-fluorouracil (B is the MFI at a given concentration and B_0 is the MFI at 0 concentration) which shows a change from 100 % B/B_0 to about 10 % B/B_0 over the concentration range 0–1000 ng/ml. If 90 % B/B_0 is used as LOD then 13.4 ng/ml is the LOD. The curve was fitted with 4-PL function and the observed versus expected concentration is shown in Figure 1b which shows a good fit over the concentration range studied.

Paclitaxel

Figure 1a shows the % B/B_0 curve for paclitaxel which shows 100 % B/B_0 to about 5% over the concentration range of 0–100 ng/ml. The LOD based on 90 % B/B_0 is 1.6 ng/ml. The correlation of observed concentration versus expected concentration based on the 4-PL function is given in Figure 1c for concentration range 0–100 ng/ml.

Doxorubicin

Figure 1a shows the % B/B_0 curve for doxorubicin which shows 100 % B/B_0 to about 6 % B/B_0 over the range of 0–2 ng/ml. The LOD based on 90 % B/B_0 is 0.019 ng/ml. The observed concentrations versus expected concentration correlations based on the 4-PL function are given in Figure 1d for concentration range 0–1 ng/ml.

Cross-reactivity

As mentioned above, cross-reactivity was evaluated by adding each drug singularly to the mixture of microspheres and antibodies for all three drugs. None of the assays showed significant change in % B/B_0 to any analyte other than its own and therefore there was no significant cross-reactivity between the analytes.

Recovery from tiles

Again the tile recovery data is for the three drug analytes being measured simultaneously. The sensitivity of an assay based method for surface contamination will depend on the assay's sensitivity and the efficiency of the sampling method. Therefore, the tiles were spiked with more than the individual assays' upper limit which was adjusted according to the measured sampling efficiency. Since the 5-fluorouracil method had a relatively higher sampling efficiency, the tiles were spiked with two times the assay range (0–1000 ng/ml) resulting in a spiking range of 0–2000 ng/tile. The paclitaxel had a relatively low sampling efficiency so the tiles were spiked

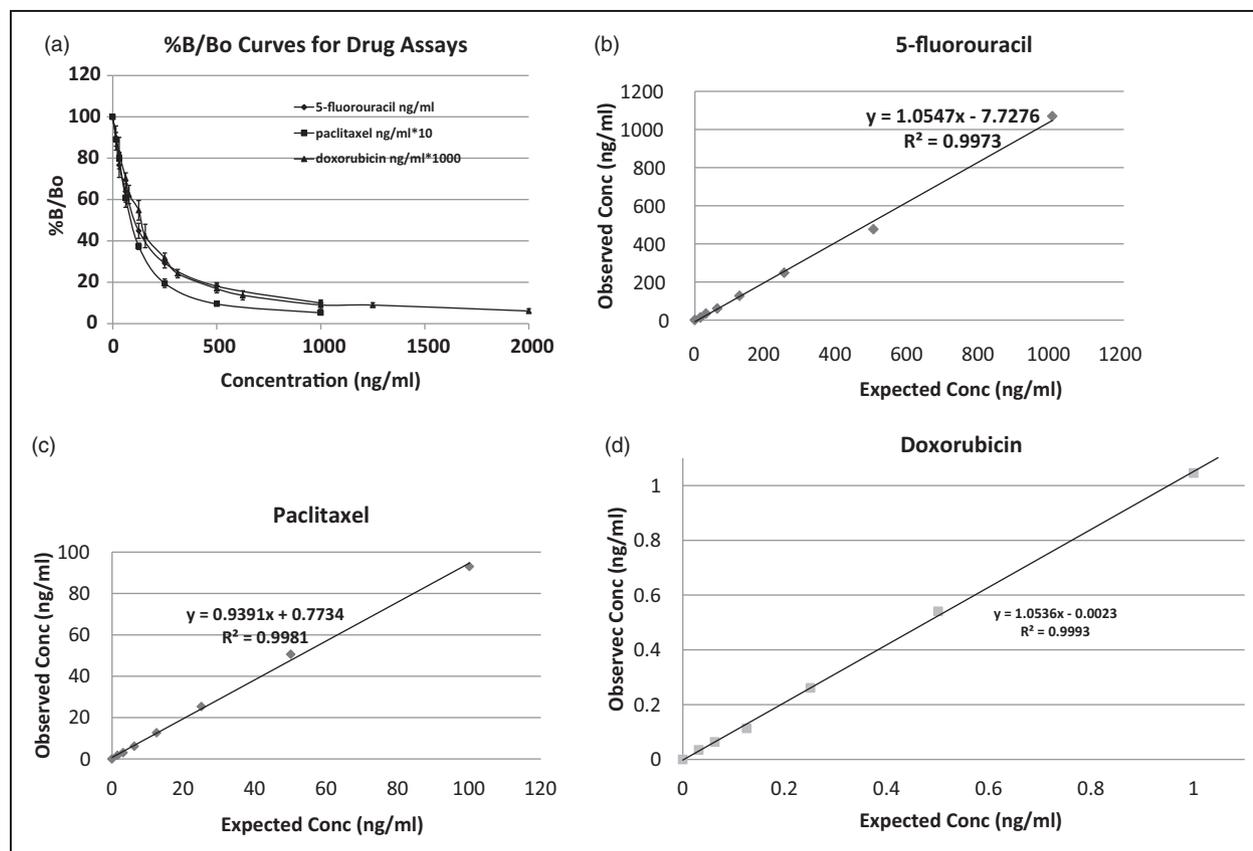


Figure 1. (a) %B/Bo for 5-fluorouracil, paclitaxel, and doxorubicin multiplex assays: B is the MFI assay signal at a given concentration and Bo is MFI assay signal at 0 concentration. Note that the range of 5-fluorouracil assay is 0–1000 ng/ml, the range for the paclitaxel assay is 0–100 ng/ml, and the range for the doxorubicin assays is 0–2 ng/ml. Errors bars are standard deviation for multiple runs. (b) Observed concentration versus expected concentration for 5-fluorouracil assay: the response curve for 5-fluorouracil shown in the figure was modeled with a four parameter logistic fit and the model was used to calculate observed concentration at each calibration point. This was plotted against expected concentration obtained from dilution factors. (c) Observed concentration versus expected concentration for paclitaxel assay: the response curve for paclitaxel shown in the figure was modeled with a four parameter logistic fit and the model was used to calculate observed concentration at each calibration point. This was plotted against expected concentration obtained from dilution factors. (d) Observed concentration versus expected concentration for doxorubicin assay: the response curve for doxorubicin shown in the figure was modeled with a four parameter logistic fit and the model was used to calculate observed concentration at each calibration point. This was plotted against expected concentration obtained from dilution factors for the 0–1 ng/ml range.

with 10 times the assay range (0–100 ng/ml) resulting in a spiking range of 0–1000 ng/tile. Doxorubicin had an intermediate sampling efficiency so the tiles were spiked with 2.5 times the assay range (0–1 ng/ml) resulting in the spiking range of 0–2.5 ng/tile. Note that the limits of detection and quantitation were calculated based on the average of all experiments.

Figure 2 shows the recovery curve for the 5-fluorouracil spiked tiles. It shows recovery that could be modeled as a line as a function of surface mass loading over the range of 0–2000 ng/tile although the relative standard deviation was larger at the higher end and overall recovery was about 34%. The estimated LOD based on blank + 3 times standard deviation of blank was 93 ng/tile (0.93 ng/cm²) and the LOQ based on

blank + 10 times standard deviation of blank was 280 ng/tile (2.8 ng/cm²).

Figure 3 shows the paclitaxel recovery over the range of 0–1000 ng/tile. The 0–1000 curve showed curvature at lower loading so the range of 0–62.5 ng/tile was also plotted. The 0–1000 range shows low recovery (about 1.4%) that can be modeled as a line over the upper range but the 0–62.5 range shows somewhat better recovery (about 2.8%). Even with the low recovery, the relative standard deviation is small over the entire 0–1000 range as shown by the small error bars. The better recovery at lower surface loading might be expected due to the low solubility of paclitaxel in buffer solution. The estimated LOD based on blank + 3 times standard deviation of blank was 57 ng/tile (0.57 ng/cm²)

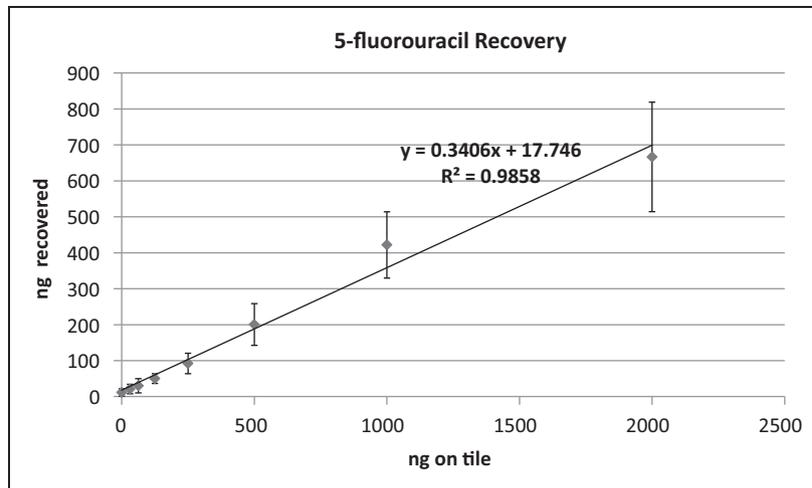


Figure 2. 5-Fluorouracil recovery from spiked tiles: tiles were spiked with 5-fluorouracil masses from 0 to 2000 ng/tile, the tiles were sampled by wiping, and the recovered mass was determined by the 5-fluorouracil multiplex assay.

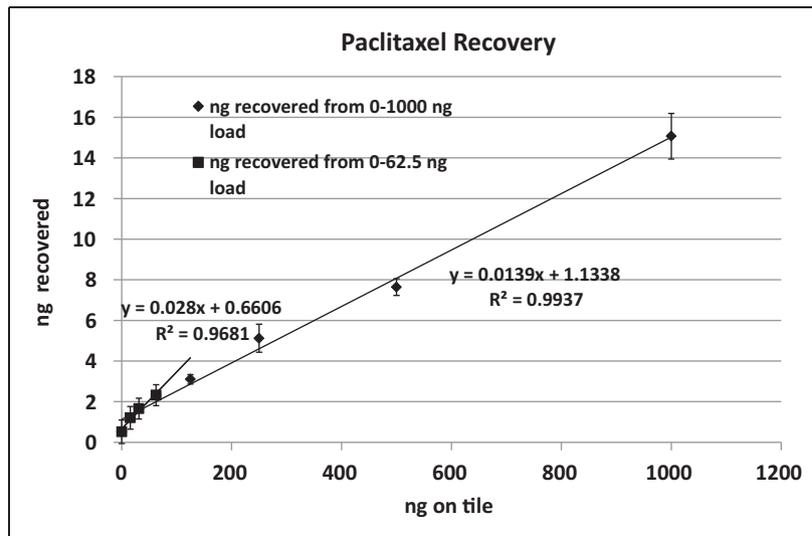


Figure 3. Paclitaxel recovery from spiked tiles: tiles were spiked with paclitaxel masses from 0 to 1000 ng/tile, the tiles were sampled by wiping, and the recovered mass was determined by the paclitaxel multiplex assay. Data are plotted for both the 0–1000 ng/tile and 0–62.5 ng/tile ranges.

and the LOQ based on blank + 10 times standard deviation of blank was 206 ng/tile (2.06 ng/cm²) based on the 0–62.5 ng/tile curve.

Figure 4 shows doxorubicin recovery (about 21%) over the range 0–2.5 ng/tile. The estimated LOD based on blank + 3 times standard deviation of blank was 0.36 ng/tile (0.0036 ng/cm²) and the LOQ based on blank + 10 times standard deviation of blank was 1.3 ng/tile (0.013 ng/cm²) based on the 0–2.5 ng/tile curve.

Discussion

Contamination of workplace surfaces by antineoplastic drugs continues to be a problem since there is evidence

that these drugs can produce exposure through skin contact.^{1,14,15} Therefore methods to determine surface contamination are needed. The multidrug assay based on FCMIA was capable of detecting and measuring three antineoplastic drugs (5-fluorouracil, paclitaxel, and doxorubicin) simultaneously with LODs less than 1 ng/cm² (100 ng/tile) and LOQs less than 5 ng/cm² (500 ng/tile). The LOD for the 5-fluorouracil recovery was close to 1 ng/cm² but it was calculated based on the average of all data for all runs and calculated LODs of individual runs were lower. The sampling technique is simple and the assay is capable of being done rapidly at relatively low cost once it is set up. The 5-fluorouracil assay was relatively insensitive compared to the other

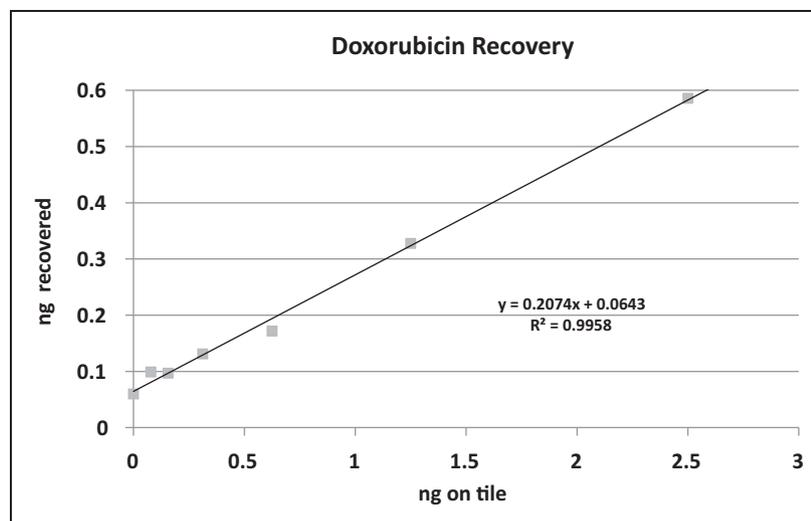


Figure 4. Doxorubicin recovery from spiked tiles: tiles were spiked with doxorubicin masses from 0 to 2.5 ng/tile, the tiles were sampled by wiping, and the recovered mass was determined by the doxorubicin multiplex assay.

assays. The same reagents were used in development of a lateral flow immunoassay which had an LOD of 5 ng/ml or less so it is not known why the FCMIA assay for 5-fluorouracil was less sensitive. The LOD of an LC-MS/MS based method for 5-fluorouracil was 0.06 ng/cm².⁶ The paclitaxel assay was more sensitive than the 5-fluorouracil assay but the wiping technique was considerably less effective at sampling the surface. Even with the low sampling efficiency the paclitaxel assay was able to detect less than 1 ng/cm². The paclitaxel recovery curve may need to be divided into several regions to cover the entire range of 0–1000 ng/tile if semi-quantitative results are desired. In this study, the curve that included all data from 0 to 1000 ng/tile fit the data from 125 ng/tile to 1000 ng/tile while the curve that only included data from 0 to 62.5 ng/tile fit the data in that range better than the overall curve. The paclitaxel sampling and analysis technique was also the most reproducible of all the assays as indicated by the small error bars. Ways of improving the recovery of paclitaxel could be studied but the modified procedure would have to be compatible with the immunoassay and be simple and convenient. The LOD of an LC-MS/MS based method for paclitaxel was 0.07 ng/cm².⁶ The doxorubicin assay is the most sensitive and it has intermediate recovery resulting in considerably lower LOD and LOQ for surface sampling than paclitaxel and 5-fluorouracil. The LOD of an LC-MS/MS based method for doxorubicin was 0.20 ng/cm².⁶ In this study surface sampling was investigated for glazed ceramic surfaces only but we have performed another study that indicated that 5-fluorouracil recovery is similar but not identical from a range of surfaces such as vinyl tiles, stainless steel, glass, and composite

using a similar sampling method and analysis with both an immunochemical and LC-MS/MS analysis method (data not shown). Further evaluation of this assay would include determining recovery from a range of surfaces in addition to ceramic tiles and other samples such as drug vials, gloves, and drug preparation mats.

The main use of this assay would be for screening surfaces in pharmacies, clinics, and drug manufacturing facilities for contamination by antineoplastic drugs. Of special interest are pharmacy areas such as hoods for drug preparation and nursing areas where drugs are administered to patients. Instrumental methods are more sensitive and specific than this assay for several of the drugs (e.g. 5-fluorouracil) but routine use of instrumental methods is expensive and samples have to be sent to a laboratory to perform the analysis which results in delays in obtaining results. Since this assay is simple, it could be set up to be performed at the worksite to provide more timely results. The components of the assay could be supplied as a kit that someone with limited training could use. Use of this assay may provide a lower cost method for routine application.

It would be desirable to add more drugs to the FCMIA assay to screen for contamination for a wider array of drugs. This would require the development of antibodies and drug–protein conjugates for the additional drugs which is a limitation of the assay. Also cross-reactivity between the drugs being measured as well as other drugs that might be present would have to be assessed. The addition of other drugs would result in minimal increases in the time to perform the assay and the FCMIA assay might be used for more drugs at a relatively low cost.

Conflict of interest

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health. Mention of company or product names does not imply endorsement by the National Institute for Occupational Safety and Health.

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