A Test Method for Assessment of Spill and Leakage From Drug Preparation Systems

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Anti-cancer drugs are reactive compounds with known adverse health effects. To prevent occupational exposure to these drugs, there are, in most countries, regulations for handling anti-cancer drugs. Many preparation systems are available, e.g. isolators, biological safety cabinets (BSCs), filter spikes (venting spikes with micro-pore filter) and closed systems (e.g. PhaSealTM). Although these systems are used, there are reports of exposure. This causes concern over how efficient these systems are to prevent spill and leakage that may cause undesired exposure when handling cytotoxic drugs. Today, this knowledge is lacking. This paper presents a method (Tc-method) for testing drug preparation systems for spill and leakage. The Tc-method is based on 99Tcm as a tracer, with which drug vials used for test preparations are spiked. Wipe samples are then collected around the working area to measure spill and leakage. The Tc-method has been validated using an independent method, showing good agreement between the methods. Spills down to 1 nl cm⁻² can be determined. In an appendix, the Tc-method is described in a detailed step-by-step procedure.

Keywords: drug leakage; preparation and administration systems; technetium; test method

INTRODUCTION

In the Swedish Medical Product Agency Ordinance Drug standard (1999) and in similar documentation in other countries, there are regulations on how drugs shall be prepared and administered to patients to ensure an aseptic and, for the patients, secure handling. There is, however, no detailed information on measures to protect the staff from undesired drug exposure. Anti-cancer and anti-viral drugs are strongly reactive and may cause adverse health effects upon exposure. In Sweden the Work Health Authority regulates by their ordinance on Cytostatics how anti-cancer drugs shall be handled and prepared in order to protect the staff (AFS Ordinance, 1999). Occupational safety authorities in other countries have similar regulations. Preparation in a Biological Safety Cabinet (BSC) has been regarded as a satisfactory method for safe preparation of these drugs. However, it has recently been discovered that drugs prepared inside BSCs may be emitted to the outside of the boxes during the preparation work. There are several reports that staff who make the preparations have had measurable levels of the prepared drugs in their blood or urine after preparation (Nygren et al., 1990, 2002; Ensslin et al., 1994; Sessink et al., 1995, 1997; Bos and Sessink, 1997; Nygren and Lundgren, 1997). It is therefore essential to evaluate the reliability of the current preparation systems (e.g. BSCs, various venting spikes with or without micro-filter as well as closed systems) and further investigate their performance.

Many anti-cancer drugs are classified carcinogens and the goal must be to eliminate the exposure as much as possible. This means that the preparation systems and procedures should be compatible to avoid spill and leakage of drugs, which may cause undesired exposure to the staff. The systems and procedures in use should therefore be tested for leakage in order to choose the best available system. Various methods to test the leakage and emission during drug preparation have been described. For example, wipe samples have been collected for determination...
of cyclophosphamide (CP) (Sessink et al., 1992; Hedmer et al., 2004) or platinum (Pt) as a tracer for cisplatin and related drugs (Nygren, 2002; Nygren and Aspman, 2004). Pt in air has also been used as a tracer for assessment of aerosols with cisplatin and related drugs in workplace air (Nygren and Lundgren, 1997; Nygren, 2002). In another approach, Kromhout et al. (2000) used the addition of a fluorescing agent in drug vials to monitor spill and leakage.

The scope of this work has been to develop an independent and objective test method to test different preparation systems and techniques regarding leakage and spill. It is desirable that the method should be applicable for evaluation of:

- different preparation techniques and systems,
- effects of various measures to decrease leakage and spill with a specific technique or system in use,
- education programs for different preparation techniques and systems,
- weak points in a preparation system and technique.

THE TEST METHOD (Tc-METHOD)

A detailed step-by-step description of the Tc-method is presented in the Appendix.

Principle of the test method

An effective test method can be obtained by utilizing a tracer substance during preparation. The size of the leakage can be determined by collecting wipe samples (Hedmer et al., 2004), on which the amount of the tracer substance is quantified. It is essential, however, that the tracer substance can be determined in a low concentration and that its toxicity is so low that the test method itself cannot pose any hazard to the test staff.

99mTc is a radioisotope with short half-life (~6 h). It is frequently used for various clinical radiotherapy investigations and detection systems to measure the radiation that is therefore already present in most hospitals. The short half-life means that the radiation rapidly declines and no residual radiation is left after a day. With a suitable amount of 99mTc as tracer, leakage in the nL cm⁻²-level can be detected without any risk of harmful radiation to the staff. The substance is readily available in hospitals and no lingering effects will remain after the test. 99mTc would therefore be suitable as tracer substance in the test system.

Considerations for the use of 99mTc

The use of 99mTc involves work with radioactive material. It is therefore necessary that the test staff receive the required instructions, for the different work operations in the test, to be able to properly handle 99mTc. A test supervisor, with adequate experience and knowledge of work with radioactive compounds, e.g. a hospital physicist, shall also be appointed for each test.

99mTc is frequently used for clinical X-ray examinations and there are investigations available, which presents measurements and calculations of the radiation dose that staff, working daily with X-ray examinations involving 99mTc, may be exposed to in their work (Ahlgren et al., 1983). Staff working full time with nuclear medical examinations (~5 patients per day for 200 working days per year) is calculated to receive a whole body dose of ~2% of the maximum radiation dose recommended by the International Commission on Radiological Protection (ICRP, 2002). The dose to individual fingers may reach the ICRP recommended maximum. The largest part of these doses occurs during patient treatment, and only a minor part occurs during preparation of the infusion solution.

During one test, each test person performs four to six preparations, which corresponds to 1 day of full time work with nuclear medical examinations. This means that the radiation dose that the test staff may be exposed to during a test is similar to that of 1 day of normal radio-physiological examination work.

Description of the Tc-method

Fifty syringes (or other equipment for administration) prepared from drug vials spiked with 99mTc should be used to test a preparation system or technique. The most relevant result is obtained if the regular staff performs these 50 test preparations. During the preparation, 10 ml of dilution solution (e.g. saline) shall be added to the vial and 6 ml withdrawn by the disposal syringe (or other equipment) used for the administration. Immediately after the preparations have been completed, wipe samples shall be collected from defined surfaces on each side of the workplace, on adjacent benches and on the floor beneath the workplace as well as on a floor surface at the other end of the room according to a previously validated procedure for collecting wipe samples (Hedmer et al., 2004). The radiation from the samples must be measured directly after sampling and the amount of collected radioactive solution on the wipe samples can then be calculated.

Validation of the Tc-method

The Tc-method was compared with an X-ray fluorescence (XRF) method (Pt-method) for determination of Pt in the wipe samples (Nygren, 2002; Nygren and Aspman, 2004) using the test protocol described in the Tc-method. Traditional preparation in BSC (with pumping or ‘milking’ technique without venting spike) was also used since this technique is in regular use and has been shown to cause leakage (Nygren et al., 2002). For the validation, 50 daily drug preparations during five working days were
made. The test drug vials used in the evaluation were real CP drug vials (Sendoxan 500 mg; Asta Medica, Germany), which were spiked with both $^{99m}$Tc ($\sim$80–100 MBq) and 500 µg Pt (Spectrascan 1.000 g l$^{-1}$; Teknolab A/S, Oslo, Norway) using a Hamilton glass syringe (500 µl; Scantec, Gothenburg, Sweden). The Pt solution was injected through the vial septum before the $^{99m}$Tc-solution was injected according to the Tc-method. Possible contamination on the outside of the vials during spiking was investigated by taking wipe samples of the spiked vials.

Five test subjects, who had not been working with CP during 1 year before the study, made the preparations. All test subjects were active working nurses with more experience or less experience of drug preparation and administration. They had all volunteered to participate and an ethics committee had approved the study regarding use of $^{99m}$Tc.

Each test person made 10 preparations each. The duration of the preparations was $\sim$20–30 min for each test person and the duration for making all 50 preparations including wipe sampling was $\sim$3–4 h. The wipe samples were collected directly after the 50 preparations were completed according to the Tc-method. Directly after the wipe samples had been collected, the radiation from the samples was measured. The mean time between spiking of the vials and the radiation measurements from the samples was $\sim$6 h.

Using the Pt-method, Pt in the wipe samples was determined using XRF (XRF 702, Niton, Billerica, MA). The instrument was operated in wipe sample mode. The wipe samples in their plastic bags were measured directly in the XRF instrument according to the instrument manual for wipe samples. Reference wipe samples (Niton) as well as in-house prepared Pt-spiked wipe tissues in plastic bags were used to verify the performance of the XRF measurements.

**RESULTS AND DISCUSSIONS**

**Validation of the test method**

The spiked vials were investigated for possible contamination on the outside during spiking by taking wipe samples from the outside of vials directly after spiking. None of the spiked vials showed any measurable activity. This result confirms that the proposed spiking technique can be used without causing any contamination on the outside of the vials.

The wipe sampling technique has been previously validated (Hedmer et al., 2004). As a complement to this validation possible cross-contamination via the plastic frame was investigated. Bench surfaces were spiked at a high spill volume. The frame was used to first sample the high volume spiked surface followed by sampling a non-contaminated surface. A low level of crossover contamination ($\leq$50 nl) could occur when a high volume spill ($>10000$ nl) was sampled before sampling a non-contaminated surface. It is therefore recommended to clean the sampling frame between each sample.

As the vials were spiked with both $^{99m}$Tc and Pt, the Tc-method was compared with the Pt-method (Nygren, 2002; Nygren and Aspman, 2004). Both the activity from $^{99m}$Tc and the amount of Pt in the samples were determined. A total of 25 vials and 25 wipe samples were analysed. With the Tc-methods contamination was found on 25 vials and in 22 wipe samples. The detection limit with the Pt-method was not as low as that with the Tc-method in these experiments. Pt, above the detection limit, was, however,
found in 16 of the 47 samples. Assuming that the added Pt is fully diluted in the volume of saline solution that was added according to the procedure in the test method, the detection limit for Pt would correspond to a leak volume of 400 nl compared to 10 nl with the Tc-method. Although the methods are not directly comparable (the Tc-method gives the collected leak volume on the tissue sample and the Pt-methods gives the amount of Pt in the same sample), the correlation between the Pt- and Tc-methods can be used for validation of the Tc-method. Fig. 1 shows the correlation between the Tc-method and the Pt-method. The linear regression algorithm obtained was

\[ y = 0.00005x - 0.0013 \]  \hspace{1cm} (1)

and the correlation coefficient was 0.999, which shows that there is a good correlation between the methods.

**CONCLUSIONS**

A test method for evaluation of drug preparation systems has been developed and validated. The method utilizes $^{99m}$Tc as a tracer of liquid drug leakage in combination with wipe sampling. Liquid spill down to 1 nl cm$^{-2}$ can be detected with a relative standard deviation (RSD) of $\leq 10\%$. The use of $^{99m}$Tc as a tracer substance, whose natural decay causes it to disappear within a day, makes this method independent and insensitive to contamination of previously used drugs and other interferences.

The possible volatility of anti-cancer drugs has recently been discussed as one route of emission (Sessink et al., 1997). The test method, described in this paper, is not, however, suitable for evaluation of gaseous drug leakage since the $^{99m}$Tc-solution has negligible volatility.

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**REFERENCES**


**APPENDIX**

**Detailed step-by-step description of the test method**

**Preparations before the test**

**Drug vials:** Due to the short half-life of $^{99m}$Tc, spiking of the drug vials must be carried out immediately before each test round is conducted. A person who has adequate education for work with radioisotopes shall carry out the spiking in a fume cupboard equipped with a lead glass window or similar radiation shielding and a separate exhaust to the outside. All solutions with activity shall be kept in lead-shielded containers.

A pertechnetate ($^{99m}$TcO$_4^-$) eluate from a $^{99m}$Tc generator (e.g. Ultra-Technekow FM, Mallinkrodt Medical or similar) with a total activity of ~5000 MBq, shall be diluted with regular saline.
solution (0.9%, w/v) to 25 ml. To 50 empty, sealed and numbered drug vials $^{99m}$Tc solution corresponding to an activity of 60–100 MBq shall be added, which is 300–500 $\mu$L of the diluted $^{99m}$Tc solution. The spike can easily be added by injection through the rubber seal utilizing a Hamilton-type glass syringe. After adding the $^{99m}$Tc solution, measure the initial activity in the vial at the time of spiking. Wipe samples shall be collected according to this method (see below) on five randomly selected spiked vials and are analysed (see below) in order to confirm that the spiking did not cause any contamination on the outside of the vials. If any of the vials shows any contamination, all spiked vials have to be investigated and contaminated vials have to be exchanged before the test can start. The vials shall be stored in a suitable lead-shielded container until they are used for the test.

**Preparation room, test surfaces and wipe samples:** Fifty disposal syringes or other equipment of the preparation system, intended for introducing dilution solution into the drug vial, shall be filled with 10 ml of saline solution. The spiked vials in the lead-shielded container and 50 sets of other necessary equipment shall be prepared and placed in a suitable place adjacent to the workspace.

Areas (100 cm$^2$) for wipe sampling shall be marked out in the preparation room. Figure A1 shows schematically how these areas can be placed. If the preparation system to be tested comprises an enclosed work area (e.g. BSC, isolator or similar), the enclosed part of the system shall be mounted and its function checked according to the manufacturers instructions. Inside the enclosure, two areas, one on each side of the work area, shall be marked out and one on the outside of the front under possible apertures for the workers hands. Furthermore, an area on the floor below the enclosure and an area on the benches or tables on each side of the enclosure shall be marked out. Finally, a larger area (750 cm$^2$) at the other side of the room shall be marked out and used to control the background level in the room. Immediately before the test begins a wipe sample shall be collected from the area intended for background control.

If the preparation system to be tested is a closed system designed to be used on an open workbench, areas shall be marked up on the bench on each side of the workspace and on the floor below the workbench. Figure A2 shows schematically how these areas can be placed. Finally, a larger area (750 cm$^2$) in the other end of the room shall be marked out and used for background control.
Analytical instrumentation: The activity measurement is made easiest at a radio-physiological department supervised by e.g. a hospital physicist. The activity in the samples shall be measured using a suitable gamma detector [e.g. a 3 × 2 inch NaI(Tl)-crystal detector (Canberra 2007P) with an integrated pre-amplifier or a similar instrumentation]. The samples shall be placed in a lead-shielded compartment at a well-defined distance from the detector. A measurement set-up like this can easily be accomplished (see Fig. A3) by placing the detector vertically on the bottom shelf of a two-storey service trolley and with the lead-shielded measuring compartment over a glass window in the top shelf just above the detector. The samples shall be placed inside the lead-shielded measuring compartment. Data collection and calculation can be made using a PC equipped with a plug-in card with a 2k multi-channel analyser (e.g. Accuspect NaI, Nuclear Data, which also can supply the necessary current to the detector, or a similar instrument). The software, enclosed with this card, can be used to measure the counts over the peak for $^{99m}$Tc (140 keV). The measuring set-up must be calibrated before the measurements are carried out.

Test supervisor, Test leader and Test persons: The test supervisor shall make sure that the staff participating in the test have the necessary knowledge for work with radioisotopes and that the work during the test is carried out according to the proper procedure for work with radioisotopes. A hospital physicist, a radiologist or similarly educated person can be a suitable test supervisor.

The test leader shall be responsible for carrying out the test. This work comprises preparations before the test, organizing the test, taking the wipe samples, measuring the activity as well as calculating and compiling the test results. The test leader must receive adequate education for these work tasks. Suitable test leader can be an experienced nurse, an occupational hygienist or similarly educated person.

The most relevant result of the test is obtained if the regular staff carries out the 50 preparations, equally divided between themselves. The test will thus reflect the variations in experience, skill and work patterns among the staff at that particular work place. If a new preparation system is tested, the test persons, not familiar with this system, must receive adequate training on the system according to the manufacturer’s recommendations and proper instructions for handling the spiked vials.

Test preparation In advance of each test person’s preparations, the test leader shall place the necessary syringes, or other equipment of the preparation system, all pre-filled with 10 ml saline solution, close to the workspace. The test leader shall also place, close to the workspace, all other necessary equipment needed for the preparations, e.g. needles, lead-shielded disposal containers etc. Finally, two kidney dishes and the number of vials to be used by the test person shall be placed close to the workspace. It is recommended that the test leader use tongs (or similar devices) to avoid unnecessary contact with the vials.

The test person, supplied with the protective equipment normally used for drug preparation, shall make the decided number of preparations in a row without any pause. Each preparation involves injection of 10 ml dilution solution into the drug vial followed by withdrawal of 6 ml solution back to the syringe or the injection device normally used for administration. The used drug vial shall be placed in one of the kidney trays and the prepared syringe (or injection device) in the other kidney tray. Between each preparation the test person shall change gloves to avoid transferring possible spill between vials. The test leader shall take notes of vial number and time of preparation of each vial and whether any visible spill occurs.

After the preparations have been completed all vials and other equipment with measurable activity should immediately be placed and stored in a lead-shielded cupboard, or similar store, until the activity has decayed. This waste can then be handled as normal laboratory hazardous waste.

Wipe samples When all 50 preparations have been carried out, wipe samples shall be collected from all marked areas. Non-woven swabs (e.g. Hartman Medicare, Sweden) or tissues of similar material and size, each wetted with 1 ml sodium hydroxide solution (0.3 mol l$^{-1}$), are suitable to use for collection of the wipe samples. A plastic frame, which encloses 100 cm$^2$, is a useful tool to facilitate a reproducible collection of the wipe samples. The whole sampling area shall carefully be wiped in two directions, up–down and left–right, using one
swab for each direction. Place both swabs in a plastic bag, seal and label the bag for proper identification of the sample. Prepare blank samples, using unused wetted swabs, in the same way. Clean the wipe sampling frame between each sample using an extra wipe wetted with the wipe solution. Use disposal gloves when collecting wipe samples and change gloves between each sample to avoid cross-contamination. Sodium hydroxide is corrosive, it is therefore recommended to use protective goggles, too.

To verify possible contamination on the outside of the spiked vials, wipe samples shall be collected on five randomly selected vials directly after spiking and also taking measurements of the initial activity. Each vial shall be carefully wiped using a swab wetted with 1 ml sodium hydroxide solution. Each swab shall then be placed in a plastic bag, sealed and labelled for proper identification. The radiation of the sample shall be measured according to the described procedure (see below).

Analyses The activity in the samples shall be measured immediately after the wipe sampling has been completed. Begin the measurements by counting pulses from the background radiation during 1000 s at the energy level corresponding to 99Tcm (140 keV). Then measure the activity in the samples one by one for a duration of 100 s each. Finally, measure the background once more.

Calculation of sampled leak volumes The leak volume collected from each wipe area can be calculated from the measured activity in the sample and the known activity in the solution in the vials. Due to the short half-life of the isotope, the decay during the time between preparation of the vial and measurement of the sample has to be considered.

During the activity measurement, for the decided measuring time the number of counts at the energy of 99Tcm (140 keV) are obtained. 99Tcm has a pulse intensity that gives an activity of 120 Bq per pulse. This means that the obtained number of pulses has to be recalculated to pulses per second and multiplied by 120 to obtain the activity expressed in (Bequerel, Bq). For practical reasons (suitable size of the numbers) the values shall be expressed as mega-Bequerel (MBq).

The relation between activity and volume is:

\[ \frac{A_0}{V_0} = \frac{A_t}{V_t}, \]  

(A1)

where \( A_0 \) and \( V_0 \) are the initial activity and volume and \( A_t \) and \( V_t \) are the activity and volume at the time \( t \). The leak volume is then:

\[ V_t = \frac{A_t V_0}{A_0}. \]  

(A2)

Due to the decay that occurs during the test, a correction of the activity has to be made. With the normal algorithm for radioactive decay, the activity at a given time can be calculated according to:

\[ A = A_0 e^{-\ln2/t_{1/2} \Delta t}, \]  

(A3)

where \( A \) is the activity after the time period \( \Delta t \) and \( A_0 \) is the measured initial activity at the time \( t = 0 \) and \( t_{1/2} \) is the half-life time or alternative:

\[ A_{-\Delta t} = A_0 e^{\ln2/t_{1/2} \Delta t}, \]  

(A4)

where \( A_{-\Delta t} \) is the activity at the time period \( \Delta t \) before the activity \( A \) is measured.

Applying this to the time conditions in the test method, by using the initial activity measured for each vial at the time of spiking \( A_S \), the activity in the vial at the test preparation \( (A_P) \) can be calculated according to equation (A3):

\[ A_P = A_S e^{-(\ln2/t_{1/2})(t_P-t_S)}, \]  

(A5)

where \( t_P \) is the time for the test preparation and \( t_S \) is the time for spiking the vial. Moreover, the measured activity in the wipe sample \( (A_M) \) can be used to calculate the activity in the sample at the collection time \( (A_A) \) according to equation (A4):

\[ A_A = A_M e^{(\ln2/t_{1/2})(t_M-t_A)}, \]  

(A6)

where \( t_M \) is the time for measuring the activity in the sample and \( t_A \) is the time when the sample is collected.

To calculate the leak volume at a wipe area, these calculations are inserted into equation (A2):

\[ V_A = \frac{A_A V_P}{A_P} = \frac{A_M e^{(\ln2/t_{1/2})(t_M-t_A)}}{A_S e^{-(\ln2/t_{1/2})(t_P-t_S)}} V_P, \]  

(A7)

where \( V_A \) is the leak volume collected in the wipe sample and \( V_P \) is the volume in the vial at the test preparation, e.g. 10 ml.

Correction for the presence of background radiation is also necessary. In order to obtain higher accuracy, the background radiation shall be measured for 1000 s and the number of pulses per second shall be calculated, and the activity expressed in (MBq) according to the samples. The activity from the background radiation shall be subtracted from the sample activity before the leak volumes are calculated.

Since the background radiation is measured as an integration of the radiation during 1000 s, the measured background radiation reflects the natural variation and can be regarded as the SD of the activity measurement. The volume corresponding to this activity can be calculated for the samples. Three times this volume is therefore suitable as the volume that corresponds to the method detection limit. This means that the detection limit of the method will vary from sample to sample due to the variation of the initial activity in the vials and variation in the
backgrounds radiation (typically 10%) at the time of the measurements. However, by using the mean value of the activity in all vials in the test and the median times for spiking and preparation, the volume detection limit ($V_D$, expressed in nl) can be estimated according to:

$$V_D = 3 \sqrt{\frac{A_{PM} \ln(2/t_1/2) (t_M - t_{BM}) V_B}{A_{PM} \ln(2/t_1/2) (t_M - t_{BM})}},$$

where $A_{PM}$ is the mean activity of all spiked vials in the test, $t_{PM}$ is the median time for spiking the vials and $t_{BM}$ is the median time for the test preparations.

Applying this estimation, the volume detection limit will be in the range of 6–14 nl per sample with a RSD of ~100%. With a wipe area of 100 cm$^2$, this means that the detection limit will be of the order of 0.1 nl cm$^{-2}$. Due to the natural variation of the background, it is suitable to decide of a lowest level of quantification that is high enough to give an RSD of the measurement $<10\%$. The lowest level of quantification of the leak volume on the wipe areas will thus be $\sim 1$ nl cm$^{-2}$. 
