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Modern dosimetric tools for ^{60}Co irradiation at high containment laboratories

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Abstract

Purpose—To evaluate an innovative photo-fluorescent film as a routine dosimetric tool during ^{60}Co irradiations at a high containment biological research laboratory, and to investigate whether manufacturer-provided chamber exposure rates can be used to accurately administer a prescribed dose to biological specimens.

Materials and methods—Photo-fluorescent, lithium fluoride film dosimeters and National Institutes of Standards and Technology (NIST) transfer dosimeters were co-located in a self-shielded ^{60}Co irradiator and exposed to γ -radiation with doses ranging from 5–85 kGy. Film dose-response relationships were developed for varying temperatures simulating conditions present when irradiating infectious biological specimens. Dose measurement results from NIST transfer dosimeters were compared to doses predicted using manufacturer-provided irradiator chamber exposure rates.

Results—The film dosimeter exhibited a photo-fluorescent response signal that was consistent and nearly linear in relationship to γ -radiation exposure over a wide dose range. The dosimeter response also showed negligible effects from dose fractionization and humidity. Significant disparities existed between manufacturer-provided chamber exposure rates and actual doses administered.

Conclusion—This study demonstrates the merit of utilizing dosimetric tools to validate the process of exposing dangerous and exotic biological agents to γ -radiation at high containment laboratories. The film dosimeter used in this study can be utilized to eliminate potential for improperly administering γ -radiation doses.

Keywords

Microbiology; radiation chemistry; radiation physics; dose-response curve; dosimetry; radiation

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Introduction

Research studies that involve infectious agents are performed in laboratories at differing biosafety levels. A biosafety level is the level of the biocontainment precautions required to handle dangerous biological agents in an enclosed facility. The levels of containment range from the lowest biosafety level 1 to the highest at level 4. Biological agents used in research conducted at high containment laboratories have significant potential to affect human health, especially at facilities where Biosafety Level 4 (BSL-4) biological research is conducted. Biosafety level 4 (BSL-4) is required for work with dangerous and exotic agents that pose a high individual risk to laboratory workers, high community risk, and for which vaccines or treatments are not commonly available.

Inactivation is a particular concern when working with agents requiring BSL-4 conditions, since the consequences of a failed inactivation could be significant. Exposure to high intensity gamma (γ)-radiation inactivates biological specimens but preserves most of their properties and characteristics. This allows subsequent analyses to be performed outside of maximum containment using specific safety protocols. In addition to viable infectious agents, like viruses and bacteria, investigators frequently employ certain components such as proteins or nucleic acids isolated from purified pathogens or infected cells or animals. Also, antisera from infected animals are often used for a variety of studies.

Although chemicals inactivation is highly effective some of these specimens would be non-functional following this process. Using γ -radiation, it is possible to inactivate residual infectivity from biological material in such a manner that protein structure and function is preserved with minimal physical destruction. Therefore, certain specimens from maximum containment facilities are subjected to high levels of γ -radiation delivered in a self-shielded ^{60}Co irradiator. Radiation doses are administered based on current research experience, dose survival tests, and documentation from previous studies. Although there has been little systematic study, this method has proven extremely safe and reliable with typical dose ranges of 20–80 kilogray (kGy) and varying with the type of biological agent and media.

While process validation is a requirement for other maximum containment laboratory sterilization equipment, such as effluent decontamination systems and autoclaves, dose validation is not currently practiced when using ionizing radiation. A properly calibrated dosimetry system would be useful to routinely validate inactivation of specimens in a self-shielded ^{60}Co irradiator. Commercially available dosimetry systems and established validation procedures are regularly used at gamma irradiation facilities employed in the food, medical device and blood irradiation industries. High containment research is different from these industries in that much higher doses are administered and instantaneous dosimeter results are required. Also, the standard procedure at maximum containment laboratories is typically to place specimens in the irradiator chamber and achieve a target dose of γ -radiation based on exposure durations calculated from manufacturer provided chamber dose values.

This study was planned to evaluate a specific dosimetry system and investigate whether manufacturer-provided chamber exposure rates could be used to accurately administer a prescribed dose to specimens. The dosimetry system was evaluated to determine if it could: (1) Provide a reliable characterization of the gamma dose actually delivered to the material being inactivated; (2) be suitable under typical high containment laboratory irradiation conditions; and (3) comply with standard dosimetric practices. Initially we determined that there were several important evaluation criteria, as follows:

Application: System must be useful over doses ranging from 20–80 kGy delivered by ^{60}Co sources.

Precision and accuracy: Must be able to achieve 95% level of confidence in dose measurements. The total uncertainty of an absorbed dose measurement should be less than the 6% value recommended in International Organization for Standardization (ISO) American Society for Testing and Materials (ASTM) Guide for Selection and Calibration of Dosimetry Systems for Radiation Processing (ISO/AST 51261).

Dose validation capabilities: Results must be useful for validating the irradiation process and records easily maintainable. Dosimeter results must be available immediately after irradiation. Stability, reliability and repeatability of the dosimeters and system are crucial. The effects of humidity, temperature, and dose fractionization on the dosimeter must be minimal, or if necessary must be compensated for.

Procurement cost: The system should be reasonable in cost to purchase and to operate.

A number of routine dosimetry techniques are commercially available according to ISO/ASTM 51261 as well as ISO Technical Report 13409. However the gamma dosimetry system has to be properly matched with actual experimental needs including the dose ranges involved, irradiation temperature conditions encountered and time required for dosimeter readout (ISO/ASTM 51261). Additionally, precision and accuracy of the results must be considered with respect to the consequences of a failed irradiation process. Several publications have described the SunnaTM Precision Point LiF Film dose response and post-irradiation stabilization characteristics (Miller et al. 2002, Murphy et al. 2003a) and the dosimeter response to different environmental conditions (Murphy et al. 2003b). These publications indicated the SunnaTM Precision Point LiF luminescence-based dosimetry system was most likely suitable for measuring delivered dose of γ -radiation under conditions typically used to irradiate biological specimens in our specific high containment laboratory setting.

Materials and methods

We evaluated a film dosimeter system and associated calibration and operation techniques available from Sunna System Corporation of Richland, Washington, in the USA. The product named ‘SunnaTM Precision Point LiF Film’ was evaluated using conditions simulating our high containment research laboratory requirements. The Sunna film dosimeter is self-developing and manufactured from photo-fluorescent lithium fluoride (LiF) that upon interaction with radiation emits green fluorescence. This process is different from the typical thermoluminescent dosimetry where stored energy is released upon heating. The Sunna film signal is fixed permanently and dosimeters can be archived. Subsequent sections describe dosimetry system components, results of calibrating the dosimetry system, and procedures used to establish traceability to a national standard.

Equipment used

The dosimetry system consisted of the following components:

- *SunnaTM Precision Point (LiF) Film Dosimeters.* SunnaTM Precision Point (LiF) Film dosimeters are packaged in foil pouches in sizes that the fluorometer reader can accept. Each dosimeter is a small (1×3 cm), thin (50 μm) polymeric film containing microcrystalline dispersion of lithium fluoride (LiF). Upon irradiation with γ -rays and electrons and excitation by light, the film emits green fluorescence

at intensities increasing almost linearly with absorbed dose. The 535-nm (green) wavelength measured with a fluorometer can be precisely correlated to dose (Murphy et al. 2003a).

- *Trilogy Fluorometer dosimeter readout instrument.* A Turner Designs Model Trilogy 7200-000 fluorometer is a photometric instrument that measures the 535-nm(green) wavelength emission value of the Sunna film dosimeters. The unit displays a digital readout in fluorometer signal units (fsu), which are proportional to the fluorescence photons counted by a green-sensitive photo-multiplier tube. The Trilogy fluorometer was obtained from Turner Designs located in Sunnyvale, California, USA.
- *Boektel Film Heat Treatment Oven.* Each green fluorescence film is not stable until it has been either allowed to cure for at least 22 h at room temperature or is heat treated. Heat treatment stabilizes the dosimeter signal and allows results to be obtained quickly. Based on study results of Murphy et al. (2003b), the optimum treatment temperature was determined to be approximately 70° C for a minimum heating of 15 min in a forced-air oven. Heating for up to 3 h did not affect the signal. A Boektel Model CCC0.5d incubator (oven) was used to heat treat film dosimeters at 70 °C for 20 min. Heat treatment also allows dosimeters to be archived for potential re-analysis at a future date. The Boektel oven was obtained from American Scientific Inc. of Portland, Oregon, USA.
- *Transfer dosimeters.* Transfer dosimeters and analytical services were provided by the NIST Ionizing Radiation Division Physics Laboratory in Gaithersburg, Maryland, USA. The standard absorbed dose range for NIST transfer dosimeters is 0.5–100 kGy which can be delivered using either ^{60}Co or ^{137}Cs γ -radiation. Each NIST transfer dosimeter consisted of a small polystyrene vial containing four thin alanine pellets (0.55 mm height, 4.55 mm diameter). NIST provided services for the measurement of calibrated alanine transfer dosimeters irradiated at our facility to agreed-upon absorbed dose levels in a prescribed geometrical arrangement. The unopened poly-styrene vials were then returned to NIST to be measured and the results certified. The alanine transfer dosimeter certifications typically have an expanded uncertainty of approximately 1.8% for absorbed dose in water.

Calibration of dosimeters

Calibration is required in order to determine the relationship between the dosimeter fluorescence response and the actual dose received by the dosimeter. We developed a calibration procedure based on principles found in 'Guidance Notes on the Dosimetric Aspects of Dose-setting Methods' (Panel on Gamma & Electron Irradiation 2001) as well as ASTM Standard E2304-03 (ASTM E2304-03) and ISO Standard 11137. The dosimetry system was calibrated over a dose range of 5.0–85.0 kGy, which is wider than its intended use. According to ASTM E2304-03, reference dosimetry calibration should involve a minimum of five equidistant dose points per calibration curve. The calibration procedure used for this study is described in general below.

1. Irradiations were performed in-house using a Model 484-R-2 ^{60}Co irradiator (manufacturer: JL Shepherd and Associates, San Fernando, CA) with a source strength of 17,100 Ci. Chamber dose rates of either 10.5 Gy/min or 25.4 Gy/min were utilized at doses nominally set at 5, 25, 45, 65 and 85 kGy. The corresponding exposure times were calculated from the chamber dose rates (provided by the manufacturer at the time of installation 20 June 2008 and reported to be accurate with $\pm 5\%$).

2. One transfer dosimeter and five replicate Sunna film dosimeters were irradiated at each equidistant dose point. Five Sunna films were attached to the exterior of each NIST vial such that each film was orthogonal to the direction of the radiation source to minimize angular dependence effects.
3. During each irradiation the films and calibration dosimeters were positioned in a consistent 'reference' position inside the irradiator chamber with dosimeters under charged particle equilibrium (CPE) on the material surface. CPE, which eliminates electron contamination produced by photon beams, was achieved by irradiating dosimeters inside a build-up jacket. Due to the ^{60}Co dual γ -ray energies of 1.07 MeV and 1.33 MeV, a cylindrical container of 4.0 mm wall thickness polypropylene was used for CPE. The build-up plate thickness was calculated using continuous slowing down approximation values for electrons with energies of the maximum 1.33 MeV in polypropylene (International Commission on Radiation Units and Measurements [ICRU] Report 14, 1969). If irradiation is performed without CPE, then results would be susceptible to slightly varying energy build-up and geometrical conditions inside the irradiator chamber.
4. For calibration studies, we chose to irradiate at three separate temperature ranges representing these conditions: Ambient room (41.5°C), wet ice (3.3°C), and dry ice average temperatures (-80°C). These conditions were selected in order to simulate the typical irradiation process for common biological specimens. The films were set atop a Styrofoam block so as to be surrounded by air with the surface of the films set perpendicular to the main axis of the ^{60}Co radiation field. Sunna film temperatures were measured just before starting irradiation and immediately after irradiation using a Supco LIT8B Infrared Thermometer. Temperature of the NIST transfer dosimeter and Sunna film dosimeters were controlled and held constant during irradiations, which required replenishing ice periodically. This resulted in short dose fractionizations of approximately 5 min or less each time ice was replenished. Based on published results (Murphy et al. 2003a) for heat-treated dosimeters, no measurable dose fractionization effects, e.g., less than 1%, were expected for interruptions of less than 30 min.
5. All irradiated Sunna films were heat treated within 15 min after irradiation and fluorescence readings were taken immediately after heat treatment. All films were heat treated at 70°C for 20 min as noted above. All fluorescence readings were corrected for background film fluorescence. Background fluorescence was determined measuring five unirradiated dosimeters that had been heated to 70°C for 20 min and calculating the mean fluorometer readout. The background fluorescence for the wet ice and dry ice calibration curves was determined using the same procedure, except the five irradiators were held at their respective temperatures for approximately 1 h before heat stabilization.
6. Upon completion of irradiation, the unopened NIST transfer dosimeters were returned to NIST to be measured and evaluated along with a control vial of the same type that was packaged separately and was not irradiated. NIST provided a written absorbed-dose measurement certificate reporting sample results and providing a description of relevant calibration information, uncertainties and other related factors.

Results

Table I presents target γ -radiation doses, the NIST absorbed dose results for transfer dosimeters, and the relative percent difference between target and actual doses. Figure 1 graphically portrays the relationship between target dose and actual dose.

NIST transfer dosimeters irradiated at ambient room temperature or while surrounded with wet ice conditions were given an expanded uncertainty of $\pm 1.9\%$ at an approximate confidence level of 95%. Transfer dosimeters irradiated while surrounded with dry ice were given an expanded uncertainty of $\pm 2.5\%$ at an approximate confidence level of 95%. NIST determination of expanded uncertainty included Type A and Type B uncertainties combined in quadrature (the square root of the sum of the squares) and multiplied by a coverage t-factor of 2.05 to yield an expanded uncertainty of $\pm 1.9\%$ and $\pm 2.5\%$ at an approximate confidence level of 95%. Type A uncertainty is standard uncertainty evaluated by statistical methods and does not include Type B uncertainties which are based on scientific judgments.

Table II presents SunnaTM film fluorescence response signals and NIST transfer standard absorbed dose results at each target dose and temperature condition. The film results are accompanied by the standard error of the mean in units of percent and fluorescence signal units (fsu).

Figure 2 presents calibration curves developed for the SunnaTM film dosimetry system at temperature conditions of 41.5°C, 3.3°C and -80°C. These calibration curves were prepared by reverse plotting the Sunna film absorbed dose-response relationship. NIST transfer dosimeter results were plotted versus the film net signal gains, i.e., fluorescence value corrected for background to determine the best fit, second order polynomial of the equation:

$$y=(a)x^2+(b)x+(c)$$

where: y = Delivered dose in kilograys from NIST transfer dosimeters, x = Sunna film mean fluorescence signal gain, and (a), (b) and (c) = constants

The analysis of Sunna film calibration curves for three temperature conditions resulted in a distinct polynomial function for each temperature. Consistent results are demonstrated for each of the three temperature conditions based on the coefficient of determination (R^2) values for each equation. The resulting R^2 value were all close to 1.0 and all calibration curves exhibited a very good data fit, which implies the calibration relationships can be accurately used to measure doses during routine irradiations in the future.

Discussion

Several publications have described the SunnaTM Precision Point LiF Film dose response and post-irradiation stabilization characteristics (Miller et al. 2002, Murphy et al. 2003a) and the dosimeter response to different environmental conditions (Murphy et al. 2003b). Sunna film dose-response and calibration curve data from our study agreed with earlier publications, the Sunna system provides high accuracy and precision, and consistently linear (average R^2 value of 0.9962) dosimeter signal over a wide dose range. Standard error of the mean (SEM) for each group of five replicate irradiated films ranged from 0.2–3.0%. Film SEM results appeared to occur randomly and were not prone to any dose point or temperature condition. The dosimeter response also shows negligible effects from the conditions of dose fractionization and humidity we used.

The differences between manufacturer-provided chamber dose values and actual doses were 7.4% on average, with a maximum difference of 17.5%. Actual doses were less than target doses in 93% of our experimental measurements. Predicted doses were often not within the $\pm 5\%$ accuracy indicated by source-calibration certifications provided by the irradiator manufacturer. Irradiation disparities that affect dose include small variations in distance from the samples to cobalt sources, variations in source travel time at beginning or end of

irradiation, and the dose rate variance for portions of the irradiated sample that are outside the centerline iso-dose contours presented on the source-calibration certifications. These disparities introduce dose uncertainties which are important because the biological specimens would undergo the same variations and could potentially receive less or more than the prescribed dose. Also, dose measurements for short exposure times appear to be prone to larger errors. This is most likely due to exposures during source travel time which has a magnified effect during short run irradiations.

A recent study performed at Duke University showed that manufacturer-provided exposure rates were up to 95% different than actual dose rates in some regions of the irradiator chamber (Brady et al. 2009). Although our comparison of calculated doses to actual dose rates did not indicate discrepancies as large as reported in the Duke study, our results did demonstrate the merit of utilizing dosimetric tools to validate the irradiation process.

We concluded that the SunnaTM LiF Film and the Trilogy fluorometer dosimetry system were easy to use and capable of producing precise and accurate results when operating under typical irradiation conditions used at our facility. The system calibration and LiF film dosimeter development results satisfactorily met guidance requirements specified in by ASTM (ISO/ASTM Standard 51261) and by the Panel on Gamma & Electron Irradiation (2001).

Dosimetry system calibration must be repeated at least annually against NIST transfer dosimeters in order to comply with ISO/ASTM Standard 51261. If irradiation and dose measurement are performed strictly according to procedures established in this study, the estimate of total uncertainty of an absorbed dose measurement should be less than the 6% value recommended in ISO/ASTM Standard 51261 for dosimetry systems able to achieve 95% level of confidence in dose measurements (ASTM Standard E1707, 1995).

In summary, our results demonstrate the merit of utilizing dosimetric tools to validate the irradiation process at high containment laboratories. Uncertainties of administered dose are present during operation of self-shielded ⁶⁰Co irradiators. The system and techniques used during this investigation would be suitable for quickly measuring dose with an acceptable level of confidence. Considering the potential consequences of an inadequate irradiation with respect to dangerous agents undergoing research at high containment laboratories irradiation process validation is worthy of incorporating into high containment laboratory standard operating procedures.

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References

- American Society for Testing and Materials (ASTM) E2304-03. Standard Practice for Use of a LiF Photo-Fluorescent Film Dosimetry System E2304-03 developed by ASTM International.
- ISO/ASTM Standard 51261. Guide for Selection and Calibration of Dosimetry Systems for Radiation Processing. American Society for Testing and Materials; 100 Barr Harbor Drive, West Conshohocken, PA 19428, USA: 1994.

- ASTM Standard E1707. Guide for Estimating Uncertainties in Dosimetry for Radiation Processing. American Society for Testing and Materials; 100 Barr Harbor Drive, West Conshohocken, PA 19428, USA: 1995.
- Brady SL, Dewhirst MW, Toncheva G, Yoshizuma TT. Characterization of a ^{137}Cs irradiator from a new perspective with modern dosimetric tools. Health Physics Society. 2009; 96:195–205.
- International Commission on Radiation Units and Measurements (ICRU). Radiation dosimetry: X-rays and gamma rays with maximum photon energies between 0.6 and 50 MeV. ICRU Report 14. 1969
- International Organization for Standardization (ISO) Standard 11137. Sterilization of health care products – requirements for validation and routine control – radiation sterilization. Geneva: ISO; 1995.
- ISO Technical Report 13409. Sterilization of health care products – radiation sterilization – substantiation of 25 kGy as a sterilization dose for small or infrequent production batches. Geneva: ISO; 1996.
- Miller SD, Kovacs A, McLaughlin W, Murphy MK, Tinker MR. Characteristics and performance of the Sunna High Dose Dosimeter using green photoluminescence and UV absorption readout methods. Radiation Protection Dosimetry. 2002; 101:53–58. [PubMed: 12382704]
- Murphy MK, Kovacs A, McLaughlin WL, Miller SD. Dose response and post-irradiation characteristics of the Sunna 535-nm photo-fluorescent film dosimeter. Radiation Physics and Chemistry. 2003a; 68(6):981–994.
- Murphy MK, Kovacs A, McLaughlin WL, Miller SD, Puhl JM. Sunna 535-nm photo-fluorescent film dosimeter response to different environmental conditions. Radiation Physics and Chemistry. 2003b; 68:995–1003.
- Panel on Gamma & Electron Irradiation, Working Group on Microbiology. Guidance Notes on the Dosimetric Aspects of Dose-setting Methods. 2001. Available from the website: http://www.irradiationpanel.org/docs/publications/dosemethods_2.pdf

Figure 1.

A plot of relative percentage difference between manufacturer-provided chamber dose rates and NIST transfer dosimeter results versus target dose. Five target dose points were measured for three distinct temperature conditions (the symbols for each are defined in the legend). Errors indicate the expanded uncertainty at a 95% confidence level.

Figure 2. Dose-response relationship for the Sunna™ film dosimetry system at three distinct temperature conditions. Calibration curve equations are shown for each temperature and symbols are defined in the legend. Errors indicate the standard error of the mean for $n = 5$ individual measurements. Errors bars are not visible when smaller than the data points.

Table I

Doses predicted based on manufacturer-provided chamber dose rates and NIST transfer dosimeter results.

Target Dose (kGy)	NIST Transfer Dosimeter (kGy)	Rel. % Diff.
Irradiation of samples at ambient room temp. (avg. temp. 41.5°C)		
5	4	-18
25	24	-5
45	43	-5
65	63	-4
85	83	-3
Irradiation of samples in wet ice (avg. temp. 3.3°C)		
5	5	-11
25	23	-7
45	39	-14
65	57	-13
85	77	-10
Irradiation of samples in dry ice (avg. temp. -80°C)		
5	5	-10
25	25	-1
45	42	-7
65	65	0.2
85	81	-5

Table II

Sunna[™] film fluorescence responses at distinct temperature conditions.

Fluorescence			
NIST Transfer Dosimeter (kGy)	Mean Net Signal (fsu)	SEM*	
		(fsu)	(%)
Irradiation of samples at ambient room (avg. temp. 41.5°C)			
0 (bg)	n/a	n/a	n/a
4	988	27	0.4
24	8,654	39	0.3
43	16,180	38	0.2
63	22,107	844	3.0
83	27,131	84	0.3
Irradiation of samples in wet ice (avg. temp. 3.3°C)			
0 (bg)	n/a	n/a	n/a
4	1,441	20	0.3
23	5,241	94	0.9
37	10,899	78	0.5
57	16,550	124	0.6
77	23,243	98	0.3
Irradiation of samples in dry ice (avg. temp. -80°C)			
0 (bg)	n/a	n/a	n/a
5	21	11	0.2
25	1,510	118	0.2
42	2,470	134	0.3
65	3,452	307	0.4
81	4,511	110	0.4

* Error indicate the standard error of the mean (SEM) for n = 5 independent measurements.