Received: 20 December 2023 | Revised: 14 August 2024 | Accepted: 6 September 2024

DOI: 10.1002/mp.17428

RESEARCH ARTICLE

MEDICAL PHYSICS

An international film dosimetry intercomparison to establish a multi-center audit framework

Sabeena Beveridge[1] | **Andrew Alves**[1] | **Mohammad Hussein**[2] | **Catharine H. Clark**[2,3,4] | **Núria Jornet**[5] | **Claudio C. B. Viegas**[6] | **Brigitte Reniers**[7] | **Paola Elisa Alvarez**[8,9] | **Godfrey Azangwe**[10] | **Krzysztof Chelminski**[10] | **Alexis Dimitriadis**[10] | **Pavel Kazantsev**[10] | **Jamema Swamidas**[10]

[1]Australian Radiation Protection and Nuclear Safety Agency, Australian Clinical Dosimetry Service, Victoria, Australia

[2]National Physical Laboratory, Middlesex, UK

[3]Radiotherapy Physics, University College London Hospital, London, UK

[4]Dept Medical Physics and Bioengineering, University College London, London, UK

[5]Servei de Radiofísica I Radioprotecció, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

[6]National Cancer Institute, Rio de Janeiro, Brazil

[7]Universiteit Hasselt, CMK, NuTeC, Diepenbeek, Belgium

[8]IROC Houston Quality Assurance Center, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

[9]Department of Radiation Physics, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

[10]Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Seibersdorf, Austria

Correspondence
Sabeena Beveridge, Australian Radiation Protection and Nuclear Safety Agency, Australian Clinical Dosimetry Service, 619 Lower Plenty Road, Yallambie, Victoria, Australia.
Email: sabeena.beveridge@arpansa.gov.au

Abstract

Background: In 2021, a Technical Meeting was hosted by the International Atomic Energy Agency (IAEA) where it was recommended that a standardized method for assessing the accuracy of film dose calculations should be established.

Purpose: To design an audit that evaluates the accuracy of film dosimetry processes. To propose a framework for identifying out-of-tolerance results and to perform an international pilot study to test the audit design.

Methods: Six members of an international Dosimetry Audit Network (DAN) developed an audit for radiochromic film dosimetry. A single host center provided the materials to each participating DAN member to conduct the audits. Materials included: (1) a set of two irradiated audit films (10 Sq: 10 cm × 10 cm, 15 Sq: 15 cm × 15 cm), (2) a reference calibration film set, and (3) a blank sheet of film. The participants were blinded to the dose and tasked with producing dose maps using their standard film dosimetry process. Average Region-Of-Interest (ROI: 2 cm × 2 cm) dose was measured from the dose maps and compared to the known dose. In the audit, all participants used their local scanning and software protocols. Film calibration was performed in two distinct ways: (1) using a calibration film set which was provided by the host center and (2) using a calibration film set which was locally irradiated. Several variations of the audit were also performed to examine how scanning and software processing can affect film dosimetry results. In the first variation of the audit (VariantA), a set of film scans was processed using five different software solutions. In the second variation of the audit (VariantB), all films were scanned on the same scanner and processed using two in-house software solutions.

Results: Taking one film scan from each participant, the standard deviations (1σ) (SD) in the dose returned from the host calibration and returned from the local calibration were ±7.2% and ±6.5% respectively, with variations from −12.4% to 12.9% of the known dose. The larger dose variations in the data set were attributed to the corrections applied for variations in scanner brightness during processing and incorrectly assigned calibration doses. When the raw image data set was processed by an expert user of each software solution (VariantA) the SDs were ±2.7% and ±3.7% for in-house and vendor-based

software, respectively. When the films were scanned on a single scanner and processed with the two in-house software solutions (VariantB) the results had a SD of ±2.3%.

Conclusions: An audit has been designed and tested for radiotherapy film dosimetry at an international level. A framework for diagnosing issues within a film dosimetry process has been proposed that could be used to audit centers that use film as a dosimeter. Incorporating quality assurance throughout the film process is important in obtaining accurate and consistent film dosimetry. A better understanding of vendor-based software systems is necessary for users to process accurate and consistent film dosimetry.

KEYWORDS
audit, film dosimetry, quality assurance

1 | INTRODUCTION

Radiochromic film is an ideal dosimeter in radiotherapy quality assurance due to its high spatial resolution while providing two-dimensional (2D) dose distributions. Some studies have characterized film uncertainty at 2.0%–3.0% (1σ) for experiments performed in the dose range of 0.8–20 Gy[1–4] in controlled dose conditions which are optimized to give the lowest uncertainty. However, it is not guaranteed that this uncertainty will be met if the dose is blind to the user of the film dosimetry system. Sub-optimal scanner[5,6] and film performance that could be detected and excluded in a controlled study may lead to increased dose uncertainty when used for clinical dosimetry. Furthermore, the end-user's level of experience in making decisions during the film processing workflow,[4,7,8] and in performing the required manual data entry, may influence the results and compromise the final dose uncertainty.

The International Atomic Energy Agency (IAEA) organized a Technical Meeting for Dosimetry Audit Networks (DANs) in August 2021, at which one of the outcomes was to develop guidance on the harmonized practice for dose measurement reporting for radiochromic film. In response, the IAEA invited experts from major dosimetry audit centers across the world, who routinely use film, to provide guidance on developing an audit for film dosimetry.

The group had three goals:

1. To design an audit that evaluates the accuracy of film dosimetry processes, regardless of equipment, software, and processing technique.
2. To run an international pilot study to test the audit design, with multiple centers that use various film dosimetry processing methods.
3. To propose a framework for diagnosing out-of-tolerance results within the scope of the film dosimetry audit.

In the context of an international dosimetry intercomparison conducted by the DAN, it is important to verify that participants qualify as audit service providers for radiotherapy departments. The removal of qualification status must be considered as one of the outcomes, and removal of a participant's status as a provider of film dosimetry would have significant implications for the provision of dosimetry services. We therefore cannot understate the importance of setting appropriate tolerances and actions against these outcomes. Important aspects that should be considered within the design of the audit are listed in Table 1.

A participant is any center or organization that requires an assessment of their radiotherapy film dosimetry process and has a well-established film dosimetry protocol. This work presents a framework that can be used to identify issues within a film dosimetry process and is not intended to provide guidance on performing film dosimetry.

2 | METHODS

Six members of the DAN participated in a pilot intercomparison which was based on the audit design components in Table 1. The members were: International Atomic Energy Agency (IAEA, Austria), National Physical Laboratory (NPL, UK), Australian Clinical Dosimetry Service (ACDS, Australia), Servei de Radiofisica I Radioproteccio (SRR, Spain), Hasselt University Centre for Environmental Sciences (CMK, Belgium), and National Cancer Institute (NCI, Brazil). The pilot intercomparison was intended to assess the viability of the audit methodology and not intended to directly assess the performance of each DAN. The data was anonymized for this paper so the members could not be identified (each member was randomly assigned as DAN1 through DAN6), and the results could be examined without bias. The design of the pilot study is shown in

TABLE 1 Components that were considered when developing the audit and the reasoning associated with each component.

Component	Reasoning
Quick and easy	Audits should not strain a center's resources and staffing. The complexity and time required to perform the audit should not deter participation.
Blind	Participants should be blind to the dose on the audit films to prevent participant's established protocols from being unduly influenced by the results.
Audit tolerance	To provide a means to trigger an investigation into a center's film dosimetry to rectify sub-optimal practice. An audit tolerance should be based on a cohort of data that is representative of the uncertainties when best practice film dosimetry is performed and that is relevant to the clinical tolerances of treatments.
Benchmarking	To provide information that the audit tolerance is sensible and achievable. Demonstration and confidence that participant's film dosimetry practice is consistent with other dosimetry providers.
Diagnostic capability This diagnostic structure is outlined in Section 2.5.	There should be mechanisms inherent within the audit process that allow for isolating potential causes of out-of-tolerance results.
Clinically aligned	The tolerances applied within the audit should be clinically aligned to the dose variations relevant to treatment requirements.
Automated and structured	The parameter space of the audit is substantial: scan, calibration, processing revision, processing software, and alternate scanner. Keeping track of the multiple versions of submitted dose maps requires a defined data structure which is addressed as the dose maps are input into the system. Establishing a well-defined data structure is essential to facilitate automation.

Figure 1. The main challenge was to construct a comparison method that was valid regardless of the processes and methodologies used by participants. The group determined that comparing a region of interest (ROI) on a participant's dose map against the known dose simplified the results and analysis, whereby this ratio could be used as a standard quality metric.

2.1 | Host and participants irradiation

A host was established to run the audit and they obtained a single box of EBT3 Gafchromic film (Ashland ISP Inc., Wayne, NJ, USA). In January 2022, the host irradiated the films for the audit on their linear accelerator (Varian True Beam v2.7). The audit films were irradiated with a 6 MV beam at a depth of 10 cm in CIRS solid water slab phantom (Norfolk, Virginia, USA). Calibration films were prepared by the host (Hostcal) for each participant and received doses of 0, 2, 4, 6, 8, and 10 Gy. Two blind films were irradiated for each participant: a 10 cm × 10 cm square field (10 Sq) and a 15 cm × 15 cm square field (15 Sq). The blind film dose was the same for each participant. The host's linear accelerator output (for each irradiation configuration) was measured with a Farmer-type PTW 30013 ionization chamber (PTW, Freiburg, Germany) directly traceable to the primary standard at BIPM (France). Only the host retained knowledge of the dose to the blind films. Irradiations for all participants were performed over 2 days after which the host distributed the films (via mail) to the participants.

Each participant received; a calibration film set, two blind dose films, and an unirradiated sheet of film (20.3 cm × 25.4 cm) from the same lot number to perform their own calibration (DANcal). The blank film sheets were distributed in advance so that synchronized calibration irradiations could be performed at the same time as the host irradiation to minimize film relative development[9–12] between host calibration and the participant's local calibration. Participants created their own calibration sets according to their protocol and used their local linear accelerator to irradiate the film, as well as used their own ionization chamber to measure dose. Participant calibrations ranged from 8 to 14 doses measured between 0 and 30 Gy. Those participants who used a larger range of calibration dose values also used higher dose sampling which ensured adequate modeling of their calibration curves. The host also kept a set of blind films so they could participate in the intercomparison by generating their own versions of the dose maps.

2.2 | Scans and dose maps

Participants were required to follow their local film dosimetry protocol, with the ability to generate 2D dose map files for submission to the audit. After a development time of at least 1 week, so that relative post-irradiation darkening between calibration films and blind films was less than 0.5%,[9] three scans (2 weeks apart) were performed for each blind film (10 and 15 Sq) and calibration film set (Hostcal and DANcal). Thus, a total of three scans were performed for each blind film which were processed with the two different calibrations, resulting in a total of six dose maps for each blind film. This was done to test the variability between scans and the variability between calibration methods. The calibration films provided by the host were also used as co-scanned reference films by the participant, if reference films were required by the participant's local film dosimetry protocol.

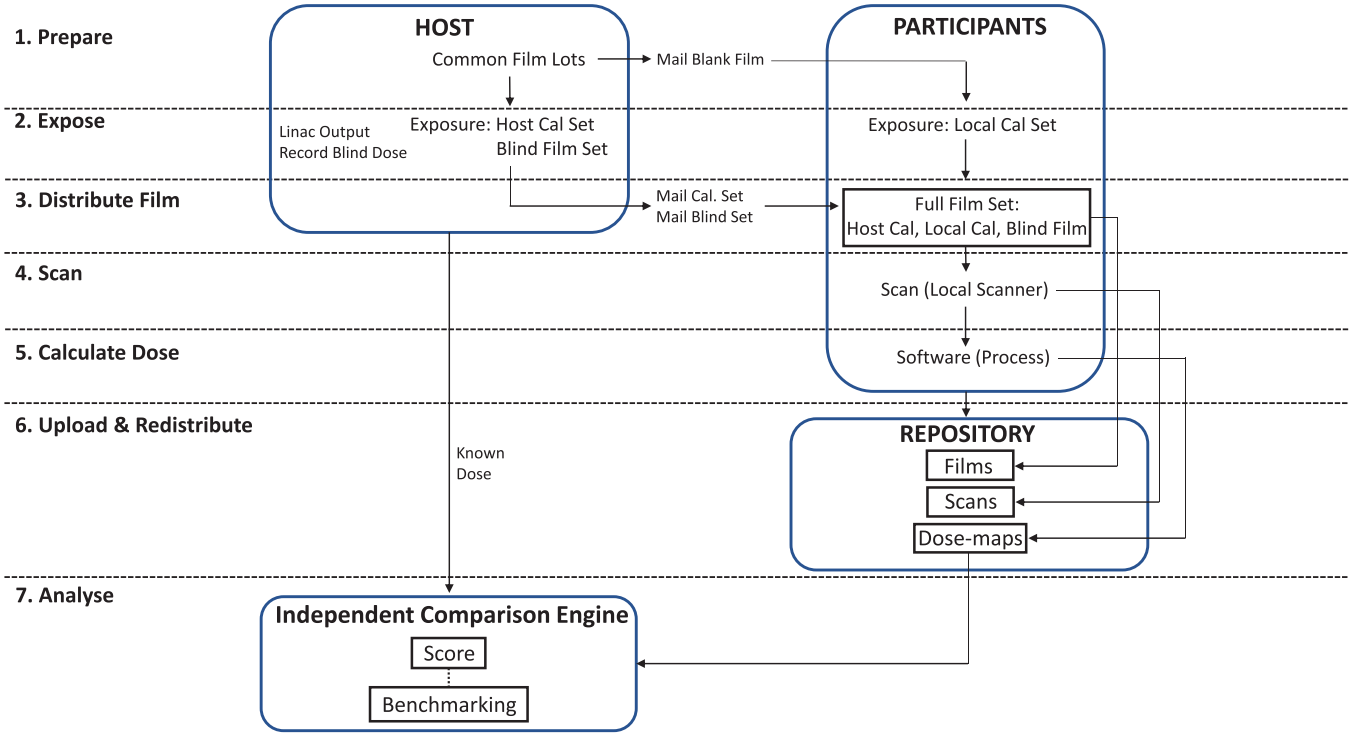

FIGURE 1 Design of a film dosimetry audit where data is continuously contributed to a repository to establish global acceptance criteria. The host is responsible for setting up the audit and is the only member that knows the dose values of the audit films.

2.3 | Repository and Independent Comparison Engine (ICE)

A subgroup of individuals from the participant pool analyzed the anonymized data which were submitted to a repository (a file server which was accessible to all participants). The digital repository containing the anonymized data served as a resource for comparing various components of the participants film dosimetry process. The data submitted consisted of the participant's calibration film sets and blind films (which were the output from the scanner, always in TIF format and recorded the pixel value of the scan) and the processed dose maps (where the file format was determined by the film dosimetry software application, and the units were in Gy). The physical films were also sent to a common location for additional scanning (as part of the repository). An Independent Comparison Engine (ICE) returned the audit metric, Q, which was the ratio of the output film dose to a 2 cm square ROI (mean value of the pixels within the ROI), D_{ROI}, to the known dose, D_{known}, which the host revealed after participants had submitted their data.

$$Q = \frac{D_{ROI}}{D_{known}} \quad (1)$$

The two values of D_{known} were: 10 Sq field $D = 5.118$ Gy, 15 Sq field $D = 5.108$ Gy. The participant's 2D film dose data files were extracted directly from the participant's film processing workflow without additional editing, smoothing, cropping, or resampling. This was so the standard quality metric present in the audit was applicable to the participant's general film based TPS verification. The output data files were considered the primary data for the audit, and they were entered using a MATLAB (MathWorks Inc., R2020a) code which was written to import the varying film dose file formats (tif, dcm, csv) that were produced by the participant's film dosimetry software.

Q was returned for each version of the submitted dose maps. Profiles were measured on the 15 Sq films inline with the scanning direction and averaged over eleven pixel-columns through the center of the irradiated field, then normalized to the known dose (D = 5.108 Gy).

The ICE was developed using MATLAB which placed an ROI (2 cm × 2 cm) at the center of the irradiated area and calculated a mean dose and standard deviation (SD) for each audit film. To keep track of the multiple versions of submitted dose maps, a defined data structure was established that recorded the scanner, scan attempt, calibration type, processing revision, and the processing software. Dose map files were directly loaded into a structured directory so that the ICE could automatically read dose maps from the primary data without the necessity for manual file selection.

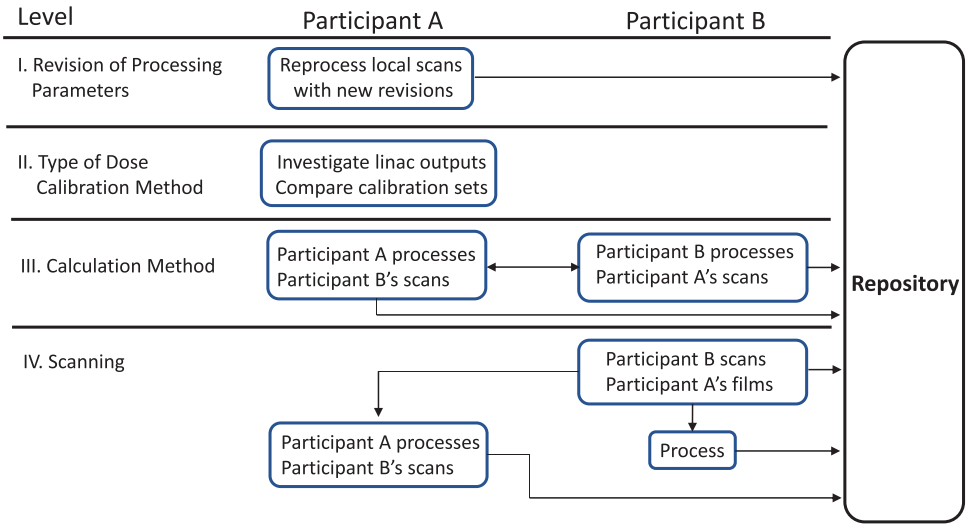

FIGURE 2 A hierarchy of assessments that can be performed if a participant fails the film dosimetry audit, with Level I being the simplest investigation and Level IV being the most involved investigation.

2.4 | Audit tolerance and outcome

Expected outcomes and tolerance values were not assigned to the pilot data due to the early stage of development of this audit methodology. Follow-up investigations were performed for every participant, as detailed in the following troubleshooting framework (Section 2.5). These follow-up investigations gave an indication of the overall uncertainty of the audit after identifiable causes of suboptimal performance had been removed. A preliminary tolerance has been suggested from the pilot results, which we propose is used to trigger troubleshooting activity in future trials of the audit methodology. We have compiled the SDs of Q across the different arms (variants) of the pilot study. These SDs are compared with the expected film dosimetry uncertainty that was drawn from previous attempts in the published literature.[1–4]

2.5 | Troubleshooting framework

The repository data was used for troubleshooting by all participants. By following the troubleshooting framework, issues were identified within each of the DAN's film dosimetry processes. To assess elements within any given film dosimetry process, a comparison hierarchy was used (shown in Figure 2).

Level I of the troubleshooting hierarchy investigated the parameters within the local software processing applications. Participants re-submitted dose maps that were generated following changes to their local dosimetry protocol. These changes included evaluating color channel processing (including multi-channel analysis), ROI size, pixel smoothing, data exclusion, and calibration fitting functions, or simply repeating the workflow to check the manual data entry.

Level II of the troubleshooting hierarchy investigated the calibration arm that was used to generate dose maps. Either the local calibration (DANcal) or the host calibration (Hostcal) set was used to obtain the calibration function. In the pilot study, every participant performed both arms of the calibration study—using both the Hostcal and the DANcal for processing.

Level III of the troubleshooting hierarchy investigated the different software processing applications used for film. Pixel value data, directly from each scanner, could be processed by any application used for film processing. In the pilot study, every participant's pixel value data was processed by several applications (as described in Section 2.6.2).

Level IV of the troubleshooting hierarchy investigated different film scanners. Physical films could be sent to a common location and scanned using a common scanner. In the pilot study, all participant's films were scanned on one participant's scanner followed by software processing using two of the available applications (as detailed in Section 2.6.2).

2.6 | Original audit and variants

The pilot study adopted the troubleshooting framework shown in Figure 2. Several variants of the original audit were performed to identify the factors which contributed to variability in the dosimetry, including scanner, calibration techniques, and software. These are summarized in Table 2 and are explained in the following sections. Table 3 presents the specifications utilized by each DAN (anonymized) in their local film processing.

TABLE 2 The list of variants performed, grouped by calibration set used for processing, scanning protocol, and processing method. Three scans were performed of each film set in the original audit. Software1 and Software2 are two different in-house software applications used for independent analysis of the films. Equivalent levels within the troubleshooting hierarchy (Figure 2) are shown in the Variant column.

Variant	Calibration used	Scanned by	Processed by	Identifier
Original audit Levels I and II	Host	DAN (x3)	DAN	Hostcal
	DAN	DAN (x3)	DAN	DANcal
VariantA: Processing Level III	Host	DAN (1st scan)	Software1	Software1
			Software2	Software2
			Vendor	Vendor1 Vendor2 Vendor3
VariantB: Scanner Level IV	Host	NPL(x1)	Software1	CommonScan_Software1
			Software2	CommonScan_Software2

TABLE 3 A comparison of the methods and components used by each DAN for their local film processing.

	Scanner type	DPI	Glass sheet	Reference films	Calibration method	Channels	Curve fitting	Software
DAN1	EPSON 12000XL	72	Yes	Yes	netOD	Multi	3rd Order polynomial	In-House
DAN2	EPSON 12000XL	150	Yes	No	netOD	Single	3rd Order polynomial	In-House
DAN3	EPSON V800	72	No	No	netOD	Multi	LN(x)	In-House
DAN4	EPSON 11000XL	96	Yes	Yes	Pixel value	Multi	Rational function[13]	Vendor
DAN5	EPSON 10000XL	72	No	Yes	netOD	Multi	Unspecified	Vendor
DAN6	EPSON 10000XL	72	Yes	Yes	netOD	Multi	Unspecified	Vendor

2.6.1 | Original audit

Each DAN was required to process the set of films from the host using their own scanner and scanning protocols, calibration methodology, and software to create a dose map. Two sets of dose maps were produced by each DAN: Hostcal using the host calibration film set, and DANcal using the DAN's calibration film set.

Co-scanned reference films (sourced from pieces of the calibration set) were used by some DANs (see Figure 3) to allow adjustment to the calibration of the unknown-dose-scan. The method of rescaling the dose of the unknown scan is dependent on the processing software that was used, user interaction of selecting regions of interest, manual entry of dose data, and choice of the reference film doses. This rescaling could potentially correct for differences in scanner constancy between the calibration scan and the unknown dose scan. Due to the irradiation schedule, the relative post-irradiation darkening between calibration films and unknown films was considered negligible, therefore, some DANs opted to scan without reference films. The dose maps created by each DAN were anonymized and sent to a central repository so a blind analysis could be performed using the ICE as described in Section 2.3.

2.6.2 | VariantA: Processing

The set of blind film scans and the Hostcal scans performed by each DAN were supplied to DAN1, DAN2, and three vendors who agreed to participate. The first scan set from each DAN (Hostcal) was used for analysis in VariantA. This variant (equivalent to a Level III assessment [see Figure 2]) was used to identify if a participant's scan could be processed using a different calculation method from the raw pixel value data.

The Hostcal and blind film scans from each DAN were processed by three vendors: FilmQAPro (Ashland, Bridgewater, NJ, USA), MyQA Patients (IBA Dosimetry, Schwarzenbruck, Germany), and Radiochromic (Radiochromic S.L., Valencia, Spain). Vendors were not told the blind dose values and they processed the film scans according to their procedure.

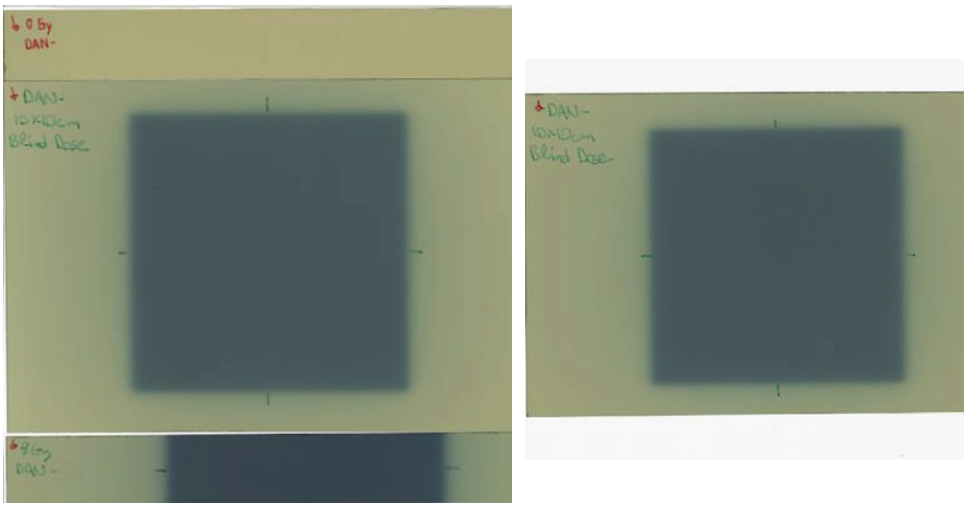

FIGURE 3 (a) An example blind dose scan co-scanned with 0 and 8 Gy reference films. Scan dose can be rescaled based on reference portions of the image. (b) Blind dose scanned with no reference films. Dose is fixed based on the scanner calibration.

The dose maps created by DAN1, DAN2, and each vendor (anonymized Vendor1, Vendor2, Vendor3) were submitted to the repository and analyzed using the ICE. The use of co-scanned references to rescale the unknown dose was left to the discretion of each operator of each software arm.

Software1

Software1 is a program developed in Python (version 3.6.5) that is used by DAN1 for film processing.[2] It uses a netOD and the calibration doses to fit a third-degree polynomial function to calibration curves in the red-green-blue (RGB) channels. Normally, a pre-scan of the calibration films (prior to irradiation) is performed when using Software1 routinely and is used to calculate the initial optical densities for the calibration films. However, there were no pre-scans performed with the host calibration film set, so the 0 Gy film was used as the initial optical density value. Adjustments of the calibration curve were performed using reference films, however, only three DANs included reference films in their scans (as listed in Table 2) which were adjusted. The DANs that do not scan with reference films did not have this scaling performed with Software1.

Software2

Software2 was developed in MATLAB 2020 and is used by DAN2 for film dosimetry.[14] Similar to Software1, it uses the netOD and known doses to fit a third-order polynomial function to generate calibration curves for the RGB channels. It does not use a pre-scan calibration film for the baseline transmission. With Software2 background measurements are taken on the scanned audit films to adjust the scan calibration.

2.6.3 | VariantB: Common scanner & software

This variant demonstrated a Level IV (see Figure 2) assessment. All the DANs sent their physical film sets to the NPL, where they were scanned on an EPSON Expression 12000XL following the NPL scanning protocol. Films were scanned in transmission mode and at a resolution of 150 dpi. A sheet of glass was placed on top of the films during scanning to ensure the films lay flat against the scanning bed.[15] Images were saved in tagged image file format (tiff) and then processed using Software1 (CommonScan_Software1) and Software2 (CommonScan_Software2). No reference films were co-scanned for VariantB.

By scanning the films on one scanner, variability between scanners was removed from the procedure. Software1 uses a multi-channel analysis and Software2 uses a single-channel analysis. For this reason, both Software1 and Software2 were used to see if software methodologies and calculations (to produce a dose map) showed a difference in results on the same scanned image.

3 | RESULTS

The proposed audit design was feasible, including the troubleshooting framework. The results were analyzed as presented below for the 6 DANs that participated. Once sorting of the data files was achieved, the development of the ICE enabled automated comparisons of all ROIs with the known dose, completing the process in a few minutes.

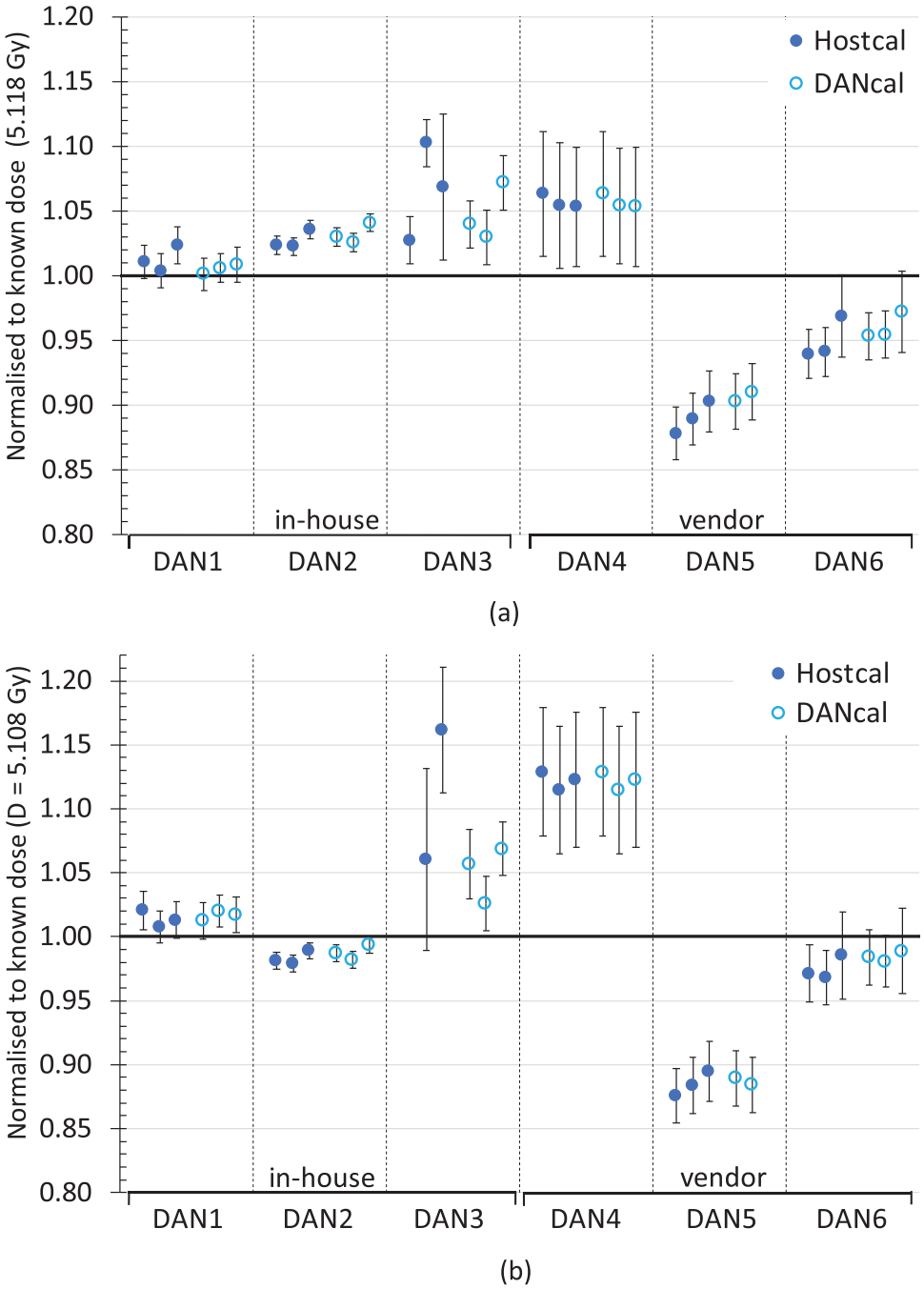

FIGURE 4 The results for the original audit—Hostcal and DANcal grouped by each DAN for the (a) 10 Sq films and (b) 15 Sq films. Data points represent the average dose value calculated within an ROI centered on the irradiated area of the film and normalized to the known dose (Q). Dose maps were created for each of the three scans performed on each film. Error bars represent one standard deviation (SD_{ROI}).

The results from the original audit and the two variants are listed in Table A1 in the Appendix.

3.1 | Original audit

The results from the original audit are shown in Figure 4. DAN5's dose maps for both the 10 and 15 Sq for their third scan (DANcal) was not provided. There was no 15 Sq dose map provided by DAN3 for their first scan using the Hostcal.

The SD within each ROI (SD_{ROI}) was used to assess the standard error in the mean of Q. The standard error in the mean for each ROI (SE_{ROI}) ranged from 0.01% to 0.12%, which was an order of magnitude in range across the DANs. Examination of the data

showed the DANs that used vendor-based software to process film dosimetry had a larger deviation from the known dose and a larger SD_{ROI} compared to those DANs that used in-house software. However, the largest SE_{ROI} that was seen was small compared to the variations in Q between different cohorts of data. Results where Q was greater than $3 \times SE_{ROI}$ can be attributed to sources of uncertainty that are not evident by assessing dose variability inside an ROI.

Comparisons between groupings of data were examined by assessing the SD of different Q values. The term SD should not be confused with SD_{ROI} where the SD is for a specific cohort of data. Based on all three scans, the Hostcal results returned an SD of ±7.4% and the DANcal results returned an SD of ±6.0%.

Combining both the 10 and 15 Sq results, discrepancies from the known dose ranged from −12.4% to 16.2%, or 4.47 to 5.94 Gy (Figure 4). Four out of the six DANs did not consistently calculate the correct dose to within ±5% for all their scans.

For all scans using the Hostcal for processing, the DANs which used in-house software returned SD = ±4.5% and DANs that used vendor software returned SD = ±8.6%. This suggests that users of vendor-based software may not be processing film in the optimal method as expected by the vendor. This prompted further investigations and the participation of the vendors to analyze the films.

In the following variants of the pilot study, only the image data from a single scan was submitted for analysis. Therefore, to provide a comparable result, the original audit returned an SD of ±7.2% and ±6.5% for the Hostcal and the DANcal, respectively, from the single scan data.

3.1.1 | Profiles

Figure 5 shows the dose profiles (inline—in the direction of the scan) for the 15 Sq films from each DAN for the original audit and the two variants: Hostcal, Software2, and CommonScan_Software2. Crossline profiles were not performed since the uncertainty across the 2 cm size of the ROI in this direction would be negligible.[2,5,13,16] As seen in Figure 5a, the profiles show not only the variation in central dose, but also the variation in the shape of the dose distribution. The profile for DAN3 in Figure 5a showed the profile inverted. Further investigation of DAN3's scan image and dose map showed that the calibration was performed incorrectly, where the high-dose calibration films were assigned low doses and the low-dose calibration films were assigned high doses. This was verified by DAN3 and by using Software1 to reproduce the inverted profile by reassigning the calibration doses (both correctly and incorrectly). This should have been seen during the processing procedure since the calibration curve would not have been characterized properly. However, DAN3's processing procedure did not include visualization of the calibration curve or quality control mechanisms in place to prevent this error. The calibration error was not immediately evident with the

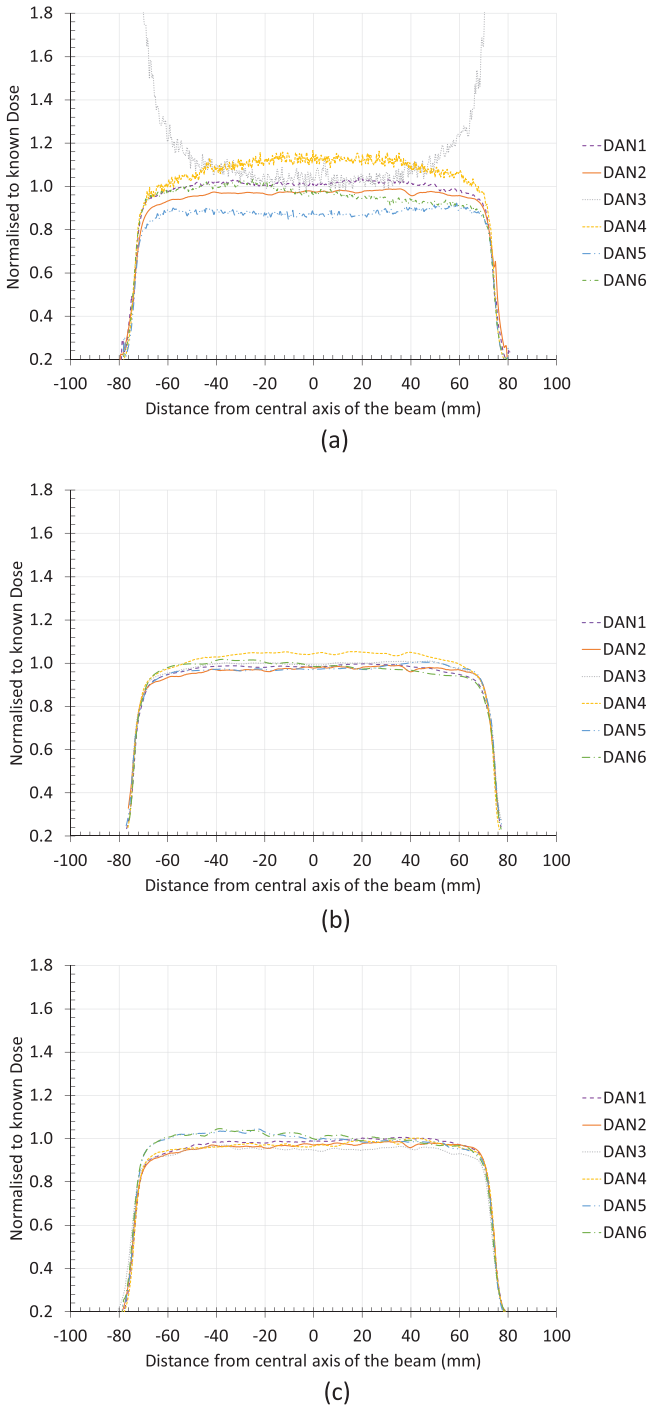

FIGURE 5 The average of the inline profiles (11 pixel-width from the center of the irradiated field) for the 15 Sq films for each DAN which was processed using the host calibration film set: (a) Hostcal, (b) Software2, and (c) CommonScan_Software2. The dose was normalized to 5.108 Gy.

ROI analysis, since the ROI was taken in the central 2 cm × 2 cm area of the irradiated film—where the audit metric, Q, was 1.060, which was inside the range of the other results that were returned in the original audit. This is partly due to the blind dose being equal to about half of the maximum calibration dose, where the calibration curves for both the correct and incorrect process would intersect.

We attribute the differences in the profiles to spatial inconsistencies between each of the participant's scanners and in each individual piece of film, since all film sets were exposed to the same field on the same Linac. Figure 5b shows greater consistency in profiles for the DANs compared to Figure 5a. This suggests that the variations seen in the profiles in Figure 5a are likely due to processing. Figure 5b,c compare the DAN-scanned films and the NPL-scanned films, respectively, where the same processing method was also used on both sets of films (Software2)—this can isolate film inconsistencies as the cause for dose variations by removing the effect of multiple scanners. A comparison of Figure 5b,c suggests that the scanners used by the DANs were not adding an appreciable level of dosimetric uncertainty relative to the variations caused by film inhomogeneities, or scanner variations within the common scanner. Figure 5c shows observable variations across the profiles for DAN5 and DAN6, which suggests these two films had similar inhomogeneities. The maximum difference in the profile dose for each DAN (within the central 80% of the profile) ranged from 5.1% to 9.2% in Figure 5c. Although the film used in this study was from a single box, there were still variations within the film that could affect the dosimetry outcomes. Developing quality control processes that can identify and correct for these issues should be considered.

3.1.2 | Revisions (Level I assessment)

Three DANs reprocessed their local scans (using the Hostcal) after the blind doses had been revealed to investigate a Level I assessment (see Figure 2) for troubleshooting. Figure 6 shows the results of the recalculated dose maps. DAN2 reprocessed their scans using a multi-channel analysis, rather than using the green channel only which is their standard protocol. The dose variation from their reanalysis was approximately 1%. DAN4 reprocessed their scans using the green channel only such that their revised results shifted by more than 5% to be within the ±5% evaluation relative to the true dose. DAN5 reprocessed their dose maps by repeating the procedure to enter the co-scanned reference dose and applying this correction. The change to the output dose in DAN5 images upon revision was up to 1% across the three scans. DAN6 reprocessed their original scan to determine if user variability made an impact on the result and verified that their processing protocol was being performed consistently across users (difference <0.1%), suggesting that further investigation may be warranted to improve their results.

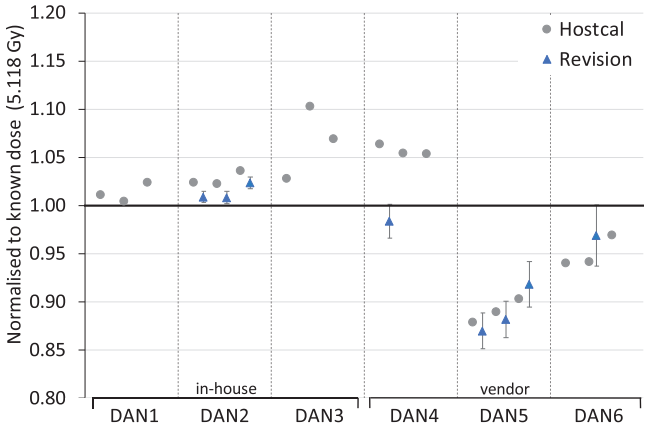

FIGURE 6 A comparison of the DANs that reprocessed their 10 Sq film results with modified techniques. The original audit results from Figure 4a are shown without error bars for comparison (Hostcal). Data points represent the average dose value calculated within an ROI centered on the irradiated area of the film and normalized to the known dose (Q). Error bars represent one standard deviation (SD_{ROI}).

3.1.3 | Calibration method (Level II assessment)

All participants processed their films using both calibration methods except for the host, who could only produce results using the Hostcal method. Each participant produced up to 6 instances of the ratio Hostcal/DANcal from the three scan attempts and the two field sizes (10 and 15 Sq). A histogram plot of the ratios for all DANs is shown in Figure 7. The mean offset was 0.13% with 77.8% of dose variations in the ±1.5% range. None of the participants exhibited a systematic difference between Hostcal and DANcal calibration methods. DAN3 produced the widest spread of results (inset 'DAN3') in comparing the two calibration methods (mean +3.5%, SD ±3.9%). This can be attributed to errors in processing the dose maps rather than the uncertainty in the participant's reference dosimetry or calibration fitting process using their chosen dose range. Further, DAN1 (inset 'DAN1') used the widest calibration range in the DANcal, (up to 30 Gy), and produced a mean agreement between the Hostcal and DANcal of +0.2% with SD = ±0.9%. There was no evidence that the range of dose values used in the calibration, or offsets in reference dosimetry between the DANs, was a major source of error. The presence of dose variations outside the ±1.5% range was attributed to other sources of error.

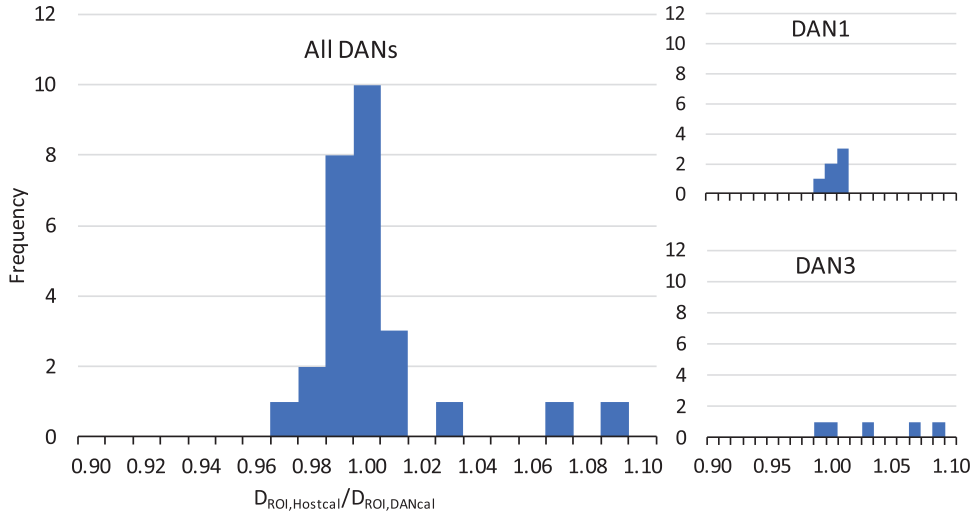

FIGURE 7 Histogram of D_{ROI} using the Hostcal divided by D_{ROI} using the DANcal for dosimetric attempts that used the same scan of the blind film. Insets show isolated data for DAN1 and DAN3.

3.2 | VariantA: Processing

3.2.1 | Software1 & Software2

The range of results presented with the original audit initiated a Level III assessment (see Figure 2) for all the participants to examine the processing methods used by each DAN to create dose maps. Figure 8 shows the dose calculated from each DAN's film scan using Software1 and Software2 to process and calibrate the 10 Sq (Figure 8a) and 15 Sq (Figure 8b) films. The results (except for DAN5, 15 Sq, Software1) were within ±5% of the known dose. The results suggest that user interaction with the software used to process the data had a significant influence on the final dose. Combining the results for 10 and 15 Sq, SD = ±2.7% when using Software1 and Software2.

For DAN5, Software1 returned a dose offset of 31.9% and 30.9% for the 10 and 15 Sq films, respectively, when no correction for co-scanned reference films was made (the result shown in Figure 8 included the reference film correction). This indicates a significant difference in constancy for the DAN5 scanner between the calibration film scan and the audit film scan. We propose that the error in the reference film correction may have been larger than a typical reference film uncertainty due to the large variation in scanner constancy.

3.2.2 | Vendors

Figure 8 also shows the results from vendor processing of the 10 Sq (Figure 8a) and 15 Sq (Figure 8b) film scans (DAN scanned film with host calibration film sets). When using all the DAN's image data, Vendor1 had the closest mean value to the reference dose for both the 10 and 15 Sq films (Q = −0.4%) and the smallest standard deviation (SD = ±2.0%), compared to Vendor2 (Q = −4.1%, SD = ±4.5%) and Vendor3 (Q = −2.2%, SD = ±2.9%). Combining the results from the three vendors for 10 and 15 Sq, resulted in Q = −2.2% and SD = ±3.7%.

The three DANs who processed their film dosimetry using vendor software in the original audit had a SD of ±9.3% in their user-processed results. In contrast, when the same image data was processed by the vendors themselves or by independent software handled by the developers, the SD results were significantly lower: ±1.7% for Vendor1, ±6.0% for Vendor2, and ±3.0% for Vendor3. This comparison highlights that user-processed film dosimetry yielded worse results than expert-processed film dosimetry, even when using identical image data.

3.2.3 | In-house and vendor

Combining the results for 10 and 15 Sq in VariantA, the SD = ±3.5% for all of the data.

3.3 | VariantB: Scanner

Results for both the 10 (Figure 9a) and 15 Sq (Figure 9b) films were within ±5% of the known dose when scanned on a single scanner (NPL) and processed using Software1 (Q = 0.1%, SD = ±1.9%) and Software2 (Q = −0.8%, SD = ±2.3%). Combining the results for Software1 and Software2 for VariantB showed SD = ±2.2%.

Comparing VariantA (DAN scanner) to VariantB (common scanner) for the two in-house software solutions we observe a reduction in SD from ±2.7% to ±2.2%.

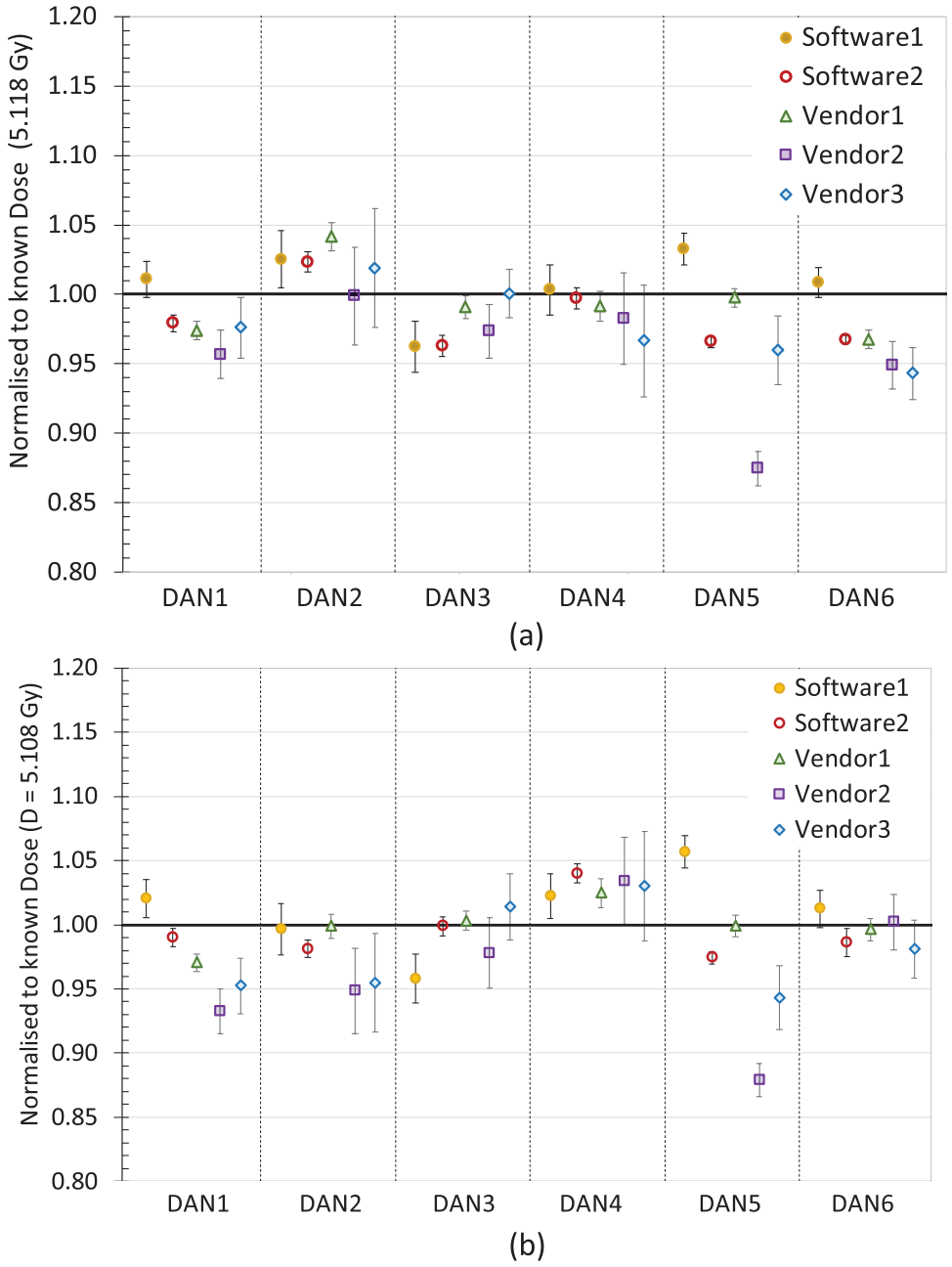

FIGURE 8 The results for VariantA—Software1, Software2, and three vendors grouped by each DAN for the (a) 10 Sq films and (b) 15 Sq films. Data points represent the average dose value calculated within an ROI centered on the irradiated area of the film and normalized to the known dose (Q). Error bars represent one standard deviation (SD_{ROI}).

This reduction could be caused by a reduced scanning uncertainty when a single scanner was used.

DAN5's results with VariantB returned a dose within 0.1% with CommonScan_Software1 and a dose within 1.8% with CommonScan_Software2. The results from this variant suggest that inconsistency in scanner response leading to a large error in reference film correction is the cause for the >5% offset seen in the original audit results for DAN5.

3.4 | Uncertainty

The audit was designed to minimize the uncertainty throughout the process where possible. Uncertainty components have been listed in Table 4. A combined uncertainty of ±2.3% has been estimated for the quality metric Q of this experiment. The participants used varying methods and procedures which included different scanners, calibration methodologies, and software for

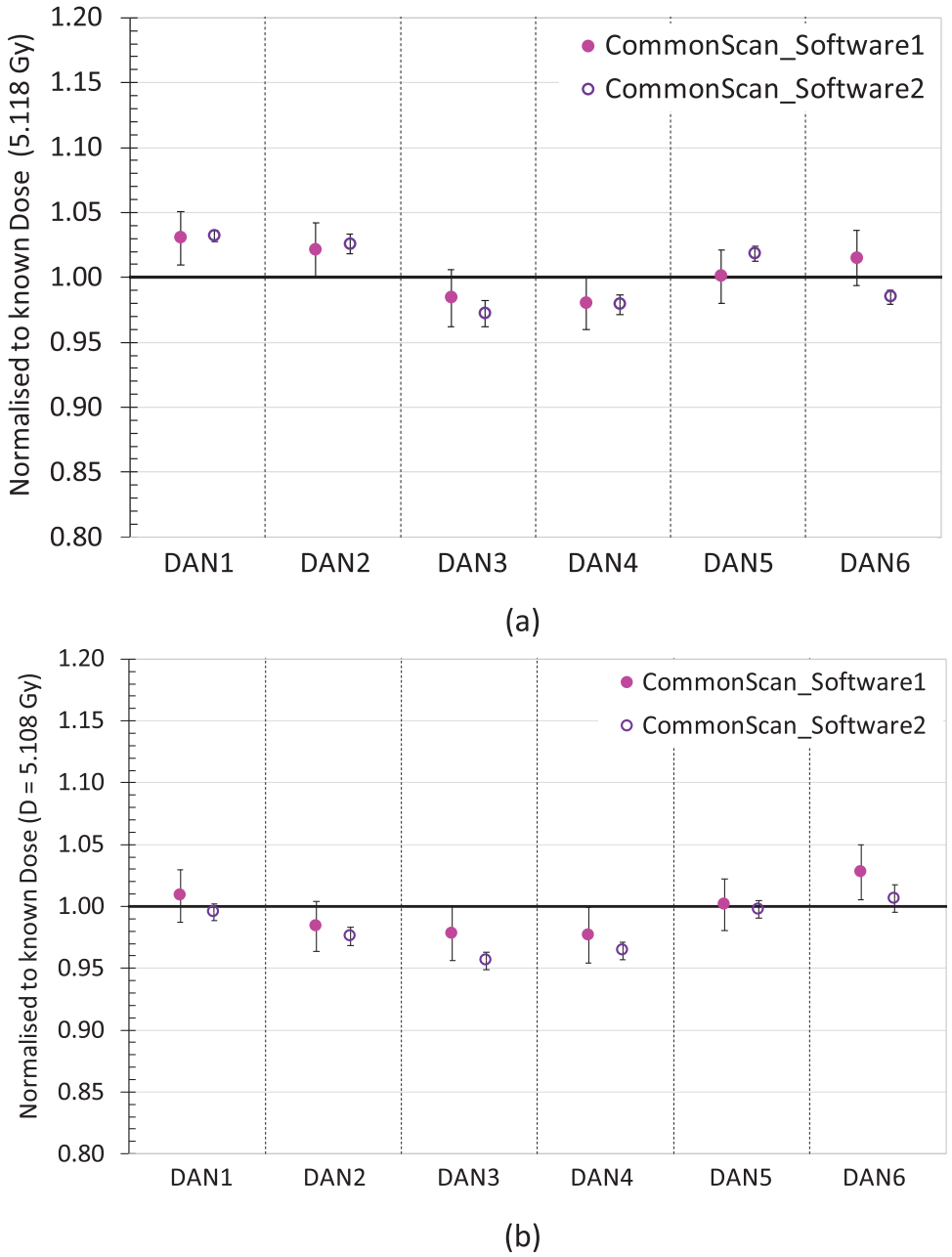

FIGURE 9 The results for VariantB—CommonScan_Software1 and CommonScan_Software2 grouped by each DAN for the (a) 10 Sq films and (b) 15 Sq films. Data points represent the average dose value calculated within an ROI centered on the irradiated area of the film and normalized to the known dose (Q). Error bars represent one standard deviation (SD_{ROI}).

processing, therefore, an estimated value was assigned across all the DANs.

All of the film was from a single box to eliminate box-to-box and lot-to-lot film uncertainty.[3,11,18] Storage conditions were kept constant, however the films used in this study were sent twice via courier and postal services to six different countries. We considered the dose uncertainty caused by transport, which includes temperature, humidity, and background radiation, to be uniform across the experiment.[19–21] There was no evidence to suggest that one participant's uncertainty was significantly altered relative to the others.

4 | DISCUSSION

The methodology for performing an international film dosimetry audit was successful in identifying sources of error in centers with film dosimetry programs, which included scanner variability, processing errors, and

TABLE 4 Components within the audit that have been considered for uncertainty analysis within the film dosimetry process. The combined uncertainty is based on the DANcal method.

Uncertainty component	(Estimated) uncertainty value	Comments
Relative dosimetry in Ion Chamber (IC) dose measurements between the host and the participant	0	In the Hostcal method, both the IC dose and the audit film dose were traceable to the same IC measurement.
	0.9%	From TRS-398,[17] in the DANcal method, the uncertainty in the IC dose of the participant and the audit film is traceable to different primary standards.
Linac stability	0.1%	The Hostcal and the audit films were irradiated in one output session and the change in Linac output can be estimated at 0.1%.
Beam flatness	0.1%	We assume that the beam is flat within the area of the ROI and consider this to be small.
Relative position of IC to film setup	0	This difference was estimated to be < 1 mm and considered negligible.
Intra-scan	1.0%	This is estimated based on the conversion of pixel value uncertainty to dose uncertainty based on a nominal calibration curve at 5 Gy.
Inter-scan	0.2%	The uncertainty in the mean dose of an ROI (at 5 Gy) due to variation in scanning attempts. Those participants who performed rescaling where reference films are co-scanned with audit films had their inter-scan uncertainty reduced to zero.
Film uniformity (audit films)	1.5%	Estimated. This is not measurable with a film scanner because it will always be coupled with scanner uncertainty.
Film uniformity (calibration films)	1.0%	The uncertainty is reduced with an increasing number of films used in the calibration.
Development conditions (Hostcal to audit films)	0	The Hostcal and audit films were irradiated at the same time. Darkening is negligible.
Development conditions (DANcal to audit films)	0.1%	Estimated. The DANcal films were irradiated within 2 days of the Hostcal films and storage conditions were similar for all DAN participants.
Software (calibration fitting)	0.1%	The measurable uncertainty in the calibration fitting is caused by scanner and film uniformity uncertainty. This is an estimated uncertainty in the interpolation between measured points.
Software (processing)	0.3%	It is unknown if triple-channel processing or single-channel processing introduced further uncertainty in this experiment. It is also unknown if rescaling with the reference film dose introduced further uncertainty in the blind dose.
Software (data entry)	–	Not possible to quantify.
Combined uncertainty	**2.3%**	

understanding software limitations. The DAN members in this study used different methodologies, techniques, and software to process film. This study has shown that quality control is important and necessary throughout the film process.

DANs showed self-consistency in scanning with dose variations less than 5% for the intra-DAN scanning attempts, except for DAN3 where scanning attempts yielded up to 10% variation in dose. It is unclear whether DAN3 could have rejected some of the scanning attempts based on the variations they observed. This emphasizes the importance of quality control and monitoring inter and intra-scan uncertainties with film scanners.[5,6,15,16,22] Participants should be encouraged to examine strategies to reject dose maps prior to submission if they find cause to do so.

One of the main aims of the study was to establish an audit design including a framework for troubleshooting out-of-tolerance results for radiotherapy film dosimetry. The audit was designed to measure a simple metric and a single dose that could be used as a standard comparison. However, simplifying the metric can cause difficulties in troubleshooting results and investigating sources of error, since only the end result is measured and not specific components throughout the process. This was evident when the original audit was performed, and the magnitude of the errors seen in two-thirds of the participants was unexpected. VariantA and VariantB demonstrated that the proposed troubleshooting framework (Figure 2) was feasible. An audit tolerance should not be determined from the SD of the original audit (±6.5% in the original audit, DANcal). Better dosimetric outcomes were demonstrated with VariantA using

common software by experienced users, therefore, an audit tolerance based on VariantA ($1\sigma = \pm 3.5\%$) should be considered for triggering follow-up investigations. More audits are required to obtain a formal uncertainty and a better-quality benchmark.

In this blind study, it was not possible for participants to use the knowledge of the expected dose to identify processing errors, therefore it highlighted the need to have quality control mechanisms in place throughout the entire process. The DANs that used in-house software to analyze their films have incorporated quality control mechanisms to identify potential errors, however, vendor-based applications need to include more options for quality measurements throughout the film process, especially where dose values can be influenced.[23]

Commercially available software that can be used to process and create dose maps offers a structured methodology for analyzing radiochromic film. Users still need to make decisions on how they will process their film: for example, single or multi-channel analysis, calibration curve fitting, and dose scaling corrections. However, there are components within these software applications that elude users—where unknown equations are being applied or the processing is occurring in the background. Comparing the original audit with the Vendor processed film scans (VariantA) for those DANs that use commercial software illustrated the importance of understanding each of the steps used in the film process. For example, DAN4 was using a triple-channel calibration process and did not realize that their software was optimized for green-channel processing, which affected their dosimetry results. DAN5 assumed their commercial software could identify scanning issues within the calibration protocol, or at least correct for it, which was not the case. Since the audit, DAN5 has changed their film dosimetry protocol to include scanner quality checks using uniform density filters prior to clinical film scanning. In-house developed software has the potential to remove these uncertainties, but most clinics do not have the resources to create, develop, and maintain bespoke software solutions.[4] This audit was able to identify issues within a film dosimetry process and assisted participants in rectifying and improving their protocols.

Further considerations must be made to accept an audit tolerance which is used to trigger any greater consequence if the methodology is developed into a formal verification tool. As for all audits, this will require a larger body of intercomparison data and a formal uncertainty budget, which is beyond the scope of this current work. We must also consider the clinical relevance of the proposed audit tolerance. Further consultation with radiation oncologists, in the context of the treatments which are being assessed in film dosimetry audits, must occur before more substantial actions against outcomes are adopted.

5 | CONCLUSIONS

This work demonstrates the importance of calibration methodology, scanning, analysis techniques, and the software used to process film dosimetry. A better understanding of vendor-based software systems is necessary for users to process accurate and consistent film dosimetry. Quality control measurements should be incorporated throughout the film dosimetry process to validate results. This work can be used in establishing a film dosimetry audit among centers who use film as a dosimeter. A larger audit cohort of baseline data is required to establish a global benchmark for achievable film dosimetry quality.

ACKNOWLEDGMENTS

The authors would like to thank the three vendors who agreed to participate in this study: FilmQAPro (Ashland, Bridgewater, NJ, USA), MyQA Patients (IBA Dosimetry, Schwarzenbruck, Germany), and Radiochromic (Radiochromic S.L., Valencia, Spain). The authors would also like to thank Raymond Sun for assisting in file and digital conversions.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

REFERENCES

1. Aland T, Kairn T, Kenny J. Evaluation of a gafchromic EBT2 film dosimetry system for radiotherapy quality assurance. *Australas Phys Eng Sci Med*. 2011;34(2):251-260. doi:10.1007/s13246-011-0072-6
2. Smyth L, Alves A, Collins K, Beveridge S. Gafchromic EBT3 film provides equivalent dosimetric performance to EBT-XD film for stereotactic radiosurgery dosimetry. *Phys Eng Sci Med*. 2024. doi:10.1007/s13246-024-01430-z
3. Marroquin EL, González JH, López MC, Barajas JV, García-Garduño O. Evaluation of the uncertainty in an EBT3 film dosimetry system utilizing net optical density. *J Appl Clin Med Phys*. 2016;17(5):466-481. doi:10.1120/jacmp.v17i5.6262
4. Palmer AL, Nash D. Radiochromic film dosimetry in radiotherapy: a survey of current practice in the United Kingdom. *Br J Radiol*. 2024;97(1155):646-651. doi:10.1093/bjr/tqae008
5. Lewis D, Chan MF. Correcting lateral response artifacts from flatbed scanners for radiochromic film dosimetry. *Med Phys*. 2016;42(1):416-429. doi:10.1118/1.4903758
6. Ferreira BC, Lopes MC, Capela M. Evaluation of an Epson flatbed scanner to read Gafchromic EBT films for radiation dosimetry. *Phys Medi Biol*. 2009;54(4):1073-1085. doi:10.1088/0031-9155/54/4/017
7. Devic S, Tomic N, Lewis D. Reference radiochromic film dosimetry: review of technical aspects. *Physica Med*. 2016;32(4):541-556. doi:10.1016/j.ejmp.2016.02.008
8. Niroomand-Rad A, Chiu-Tsao ST, Grams MP, et al. Report of AAPM task group 235 Radiochromic film dosimetry: an update to TG-55. *Med Phys*. 2020;47(12):5986-6025. doi:10.1002/mp.14497
9. Liu K, Jorge PG, Tailor R, Moeckli R, Schuler E. Comprehensive evaluation and new recommendations in the use of Gafchromic EBT3 film. *Med Phys*. 2023;50(11):7252-7262. doi:10.1002/mp.16593
10. Dreindl R, Georg D, Stock M. Radiochromic film dosimetry: considerations on precision and accuracy for EBT2 and EBT3

type films. *J Med Phys*. 2014;24(2):153-163. doi:10.1016/j.zemedi.2013.08.002
11. Reinhardt S, Hillbrand M, Wilkens JJ, Assmann W. Comparison of Gafchromic EBT2 and EBT3 films for clinical photon and proton beams. *Med Phys*. 2012;39(8):5257-5262. doi:10.1118/1.4737890
12. Palmer A, Nash D, Polak W, Wilby S. Evaluation of a new radiochromic film dosimeter, Gafchromic EBT4, for VMAT, SABR and HDR treatment delivery verification. *Phys Med Biol*. 2023;68(17):175003. doi:10.1088/1361-6560/aceb48
13. Lewis D, Micke A, Yu X, Chan MF. An efficient protocol for radiochromic film dosimetry combining calibration and measurement in a single scan. *Med Phys*. 2012;39(10):6339-6350. doi:10.1118/1.4754797
14. Hussein M, Clark CH, Nisbet A. Challenges in calculation of the gamma index in radiotherapy—towards good practice. *Physica Med*. 2017;36:1-11. doi:10.1016/j.ejmp.2017.03.001
15. Palmer AL, Bradley DA, Nisbet A. Evaluation and mitigation of potential errors in radiochromic film dosimetry due to film curvature at scanning. *J Appl Clin Med Phys*. 2015b;16(2):425-431. doi:10.1120/jacmp.v16i2.5141
16. Schoenfeld AA, Wieker S, Harder D, Poppe B. The origin of the flatbed scanner artifacts in radiochromic film dosimetry—key experiments and theoretical descriptions. *Phys Med Biol*. 2016;61(21):7704-7724. doi:10.1088/0031-9155/61/21/7704
17. International Atomic Energy Agency. Absorbed Dose Determination in External Beam Radiotherapy, Technical Reports Series No. 398 (Rev. 1), IAEA, Vienna. 2024. doi:10.61092/iaea.ve7q-y94k
18. Mizuno H, Takahashi Y, Tanaka A, et al. Homogeneity of Gafchromic EBT2 film among different lot numbers. *J Appl Clin Med Phys*. 2012;13(4):198-205. doi:10.1120/jacmp.v13i4.3763
19. Girard F, Bouchard H, Lacroix F. Reference dosimetry using radiochromic film. *J Appl Clin Med Phys*. 2012;13(6):339-353. doi:10.1120/jacmp.v13i6.3994
20. Reinstein LE, Gluckman GR. Optical density dependence on postirradiation temperature and time for MD-55-2 type radiochromic film. *Med Phys*. 1999;26(3):478-484. doi:10.1118/1.598538
21. Rink A, Lewis DF, Varma S, Vitkin A, Jaffray DA. Temperature and hydration effects on absorbance spectra and radiation sensitivity of a radiochromic medium. *Med Phys*. 2008;35(10):4545-4555. doi:10.1118/1.2975483
22. Miura H, Ozawa S, Okazue T, Enosaki T, Nagata Y. Characterization of scanning orientation and lateral response artifact for EBT4 Gafchromic film. *J Appl Clin Med Phys*. 2023;24(8). doi:10.1002/acm2.13992
23. Pocza T, Zongor Z, Melles-Bencsik B, Tatai-Szabo DZ, Major T, Pesznyak C. Comparison of three film analysis softwares using EBT2 and EBT3 films in radiotherapy. *Radiol Oncol*. 2020;54(4):505-512. doi:10.2478/raon-2020-0049

How to cite this article: Beveridge S, Alves A, Hussein M, et al. An international film dosimetry intercomparison to establish a multi-center audit framework. *Med. Phys.*. 2024;51:9071–9087. https://doi.org/10.1002/mp.17428

APPENDIX

TABLE A1 The results for the variants listed in Table 2 for each of the DANs and both the 10 and 15 Sq films. Values (±standard deviation) are normalized to the known dose: 5.118 Gy for the 10 Sq film and 5.108 Gy for the 15 Sq film. Values in () represent the scan number.

	Hostcal (1)		Hostcal (2)		Hostcal (3)		DANcal (1)		DANcal (2)		DANcal (3)	
	10 Sq	15 Sq	10 Sq	15 Sq	10 Sq	15 Sq	10 Sq	15 Sq	10 Sq	15 Sq	10 Sq	15 Sq
DAN1	1.011 ± 0.013	1.021 ± 0.015	1.004 ± 0.012	1.007 ± 0.013	1.023 ± 0.014	1.013 ± 0.014	1.001 ± 0.012	1.012 ± 0.014	1.006 ± 0.011	1.020 ± 0.012	1.009 ± 0.013	1.017 ± 0.014
DAN2	1.024 ± 0.007	0.981 ± 0.007	1.023 ± 0.007	0.979 ± 0.007	1.036 ± 0.007	0.989 ± 0.006	1.030 ± 0.007	0.987 ± 0.007	1.026 ± 0.007	0.982 ± 0.007	1.041 ± 0.007	0.993 ± 0.006
DAN3	1.028 ± 0.018	–	1.103 ± 0.069	1.060 ± 0.071	1.069 ± 0.056	1.162 ± 0.049	1.040 ± 0.018	1.057 ± 0.027	1.030 ± 0.021	1.026 ± 0.021	1.072 ± 0.021	1.069 ± 0.021
DAN4	1.063 ± 0.048	1.129 ± 0.050	1.054 ± 0.045	1.115 ± 0.050	1.054 ± 0.046	1.123 ± 0.053	1.063 ± 0.048	1.129 ± 0.050	1.054 ± 0.045	1.115 ± 0.050	1.054 ± 0.046	1.123 ± 0.053
DAN5	0.878 ± 0.020	0.876 ± 0.021	0.889 ± 0.021	0.884 ± 0.022	0.903 ± 0.024	0.895 ± 0.024	0.903 ± 0.021	0.889 ± 0.022	0.910 ± 0.022	0.884 ± 0.022	–	–
DAN6	0.940 ± 0.019	0.971 ± 0.022	0.941 ± 0.019	0.968 ± 0.021	0.969 ± 0.032	0.985 ± 0.034	0.953 ± 0.018	0.984 ± 0.021	0.954 ± 0.018	0.981 ± 0.020	0.972 ± 0.031	0.989 ± 0.033

	Software1		Software2		Vendor1		Vendor2		Vendor3		CommonScan_Software1		CommonScan_Software2	
	10 Sq	15 Sq	10 Sq	15 Sq	10 Sq	15 Sq	10 Sq	15 Sq	10 Sq	15 Sq	10 Sq	15 Sq	10 Sq	15 Sq
DAN1	1.011 ± 0.013	1.020 ± 0.015	0.979 ± 0.005	0.990 ± 0.007	0.974 ± 0.007	0.970 ± 0.007	0.957 ± 0.018	0.933 ± 0.017	0.976 ± 0.022	0.952 ± 0.022	1.030 ± 0.021	1.009 ± 0.021	1.032 ± 0.004	0.995 ± 0.007
DAN2	1.025 ± 0.021	0.996 ± 0.020	1.023 ± 0.007	0.981 ± 0.007	1.042 ± 0.010	0.999 ± 0.009	0.999 ± 0.035	0.949 ± 0.033	1.019 ± 0.043	0.955 ± 0.038	1.021 ± 0.021	0.984 ± 0.020	1.026 ± 0.008	0.976 ± 0.008
DAN3	0.962 ± 0.019	0.958 ± 0.019	0.963 ± 0.008	0.999 ± 0.007	0.991 ± 0.008	1.003 ± 0.007	0.973 ± 0.019	0.978 ± 0.028	1.000 ± 0.017	1.014 ± 0.026	0.984 ± 0.022	0.978 ± 0.022	0.972 ± 0.010	0.956 ± 0.007
DAN4	1.003 ± 0.018	1.022 ± 0.017	0.997 ± 0.008	1.040 ± 0.007	0.991 ± 0.011	1.025 ± 0.011	0.983 ± 0.033	1.034 ± 0.034	0.967 ± 0.040	1.030 ± 0.043	0.980 ± 0.021	0.977 ± 0.022	0.979 ± 0.008	0.964 ± 0.007
DAN5	1.033 ± 0.012	1.057 ± 0.013	0.966 ± 0.004	0.974 ± 0.005	0.998 ± 0.007	0.999 ± 0.008	0.874 ± 0.012	0.879 ± 0.013	0.959 ± 0.025	0.943 ± 0.025	1.001 ± 0.020	1.002 ± 0.021	1.018 ± 0.006	0.998 ± 0.007
DAN6	1.009 ± 0.011	1.012 ± 0.015	0.967 ± 0.003	0.986 ± 0.011	0.968 ± 0.007	0.996 ± 0.009	0.950 ± 0.017	1.002 ± 0.021	0.943 ± 0.019	0.981 ± 0.023	1.015 ± 0.021	1.028 ± 0.022	0.985 ± 0.005	1.006 ± 0.011