



## Direct comparison of gamma, electron beam and X-ray irradiation effects on single-use blood collection devices with plastic components

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### ABSTRACT

Effective sterilization methods for single-use devices are a growing need for the medical industry. Concerns with safety, throughput and source availability, however, prompt prudent contingency planning for gamma irradiation of devices suited for radiation sterilization. Electron beam (e-beam) and X-ray represent two alternatives to gamma radiation if they can be confirmed to be compatible with sterilization of the devices. To address this question, the effects of sterilization-relevant doses of e-beam and X-ray radiation are directly compared to the effects of gamma radiation using two prototypical commercial devices currently sterilized using cobalt-60 gamma radiation. These devices include components that comprise six distinct polymer materials commonly used in the medical device industry. The devices investigated are the Becton, Dickinson and Company (BD) *Vacutainer™ Plus* tube, comprised of low-density polyethylene, chlorobutyl rubber, and polyethylene terephthalate components; and the *BD Vacutainer™ Push Button Blood Collection Set*, containing polypropylene, polyolefin elastomer, and polyvinyl chloride components. Changes in functionality, discoloration and select mechanical properties of components of each device were measured following exposure to targeted doses of 15, 35, 50 and 80 kGy. A statistical analysis was performed to determine if the effects of e-beam or X-ray radiation differ from the effects of gamma radiation for the properties considered. No devices were found to fail the functional performance tests at any of the doses considered. Small, but statistically significant differences were observed in device discoloration from e-beam, X-ray and gamma radiation following processing for certain materials at certain dose levels. Both e-beam and X-ray irradiation appear as viable alternatives to gamma irradiation for sterilization of the medical devices and materials considered.

### 1. Introduction

Thousands of tons of medical devices are used in the U.S. every year (CDC, 2020). Most devices must be free of microbes (bacteria and viruses) and spores to avoid infection and other adverse health effects. The current breakdown of sterilization methods (modalities) used by the medical industry is 50% ethylene oxide gas, 41% cobalt-60 gamma radiation (cobalt-60 being the most common radioactive isotope of cobalt), 4.5% electron-beam (e-beam) radiation, and <5% other (including steam and X-ray radiation) (GIPA IIA, 2017). The need for

low-temperature sterilization has continued to increase with increased use of plastics in recent decades. Over 40% of single-use devices are polymer-based and can be sterilized via radiation.

Switching from gamma to e-beam or X-ray for medical device sterilization is encouraged by growth in regulations governing cobalt-60 commercial use, limitations in the cobalt-60 supply chain, and by the relatively long cycle time required for cobalt-60 sterilization. The potential ability to integrate e-beam or X-ray sterilization directly into the manufacturing process is another advantage of these gamma alternatives. A recent report by Fermilab (Kroc et al., 2018), however,

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highlights that “...there is a knowledge gap in how the different radiation sources (viz., cobalt-60, e-beam and x-ray irradiation) affect common medical device materials. Because of this, irradiation effects on materials for all three modalities need to be documented in peer-reviewed references and made publicly available to encourage use of different irradiation modalities.” This work is part of an effort to lower barriers to use of non-radioisotope methods in sterilization and to determine whether e-beam or X-ray radiation is suitable to replace gamma radiation sterilization for key medical polymers and devices.

Use of e-beam or X-ray radiation as alternative modalities to gamma radiation should be feasible based on regulatory and theoretical physics considerations. ISO 11137-1 (2013) uses the terminology of ‘transference of maximum acceptable dose’ (clause 8.4.1) to address moving from one radiation sterilization modality to another. The ‘maximum acceptable dose’ is the maximum dose at which the performance of a product has been qualified throughout its intended shelf-life. It is a requirement of the standard that the validity of the maximum acceptable dose with the new modality be assessed and documented. Guidance for this requirement (A.8.4.1, ISO 11137-1, 2013) includes, “A product qualified at a low dose rate (gamma rays) or intermediate dose rate (X-rays) will typically require minimal qualification to demonstrate material compatibility at a high dose rate (electron-beam).” This guidance is based on the essential equivalence of the three modalities apart from dose rate and temperature during irradiation. This in turn is based on the physics of energy deposition between the three modalities, i.e., all three ultimately affect materials largely through coulombic interactions of electrons with the materials (e-beam accelerators generate the electrons directly; gamma and X-ray generate the electrons through a process called Compton Scattering). In summary, a reasonable hypothesis is that the maximum acceptable dose of a product qualified at a low dose rate using gamma sterilization will be validly transferred to an e-beam or X-ray sterilization process that delivers the same dose at higher dose rates. This work provides a rigorous example in support of this hypothesis.

Conditions during the manufacturing process of high volume, polymer-based medical devices result in low microbe populations or bioburden in the finished products. However, a minimum radiation dose in the range of 15–50 kGy is still required for sterilization of most devices (AAMI TIR17, 2017). Doses in the tens of kGy can involve significant chain scission and cross-linking in polymers. Chain scission can result in reduced tensile strength and reduced elongation before break. Cross-linking can increase tensile strength but reduce elongation. Thermo-oxidative reactions initiated by radiation can also lead to yellowing and discoloration in polymers through formation of chromophores in the polymer matrix. Because of the potential impacts of sterilization dose levels on the polymers in device components, manufacturers must be cognizant of the impact of sterilization radiation on device performance.

To directly compare the effects of sterilization-relevant e-beam and X-ray radiation doses with the effects of industry-standard gamma radiation on representative medical devices, two devices were selected that are produced in high volumes, are composed of multiple polymers common to the industry, and are currently sterilized using cobalt-60 gamma radiation. The Becton, Dickinson and Company (BD) *Vacutainer*<sup>TM</sup> tube represents over 90% of the world market of blood collection tubes, with more than 5 billion units produced each year. It is composed of a polyethylene terephthalate (PET) tube, a chlorobutyl rubber (CIIR) stopper, and a low-density polyethylene (LDPE) cap. The BD *Vacutainer*<sup>TM</sup> *Push Button Blood Collection Set* is produced at a volume of 260 million units per year and includes a polypropylene homopolymer (PPH) finger grip, polyolefin elastomer (POE) control wings, and polyvinyl chloride (PVC) flexible tubing. Batches of these devices were exposed to common target doses of 15, 35, 50 and 80 kGy using all three radiation modalities. The doses were selected to envelope both sterilization doses (15–30 kGy) and the higher doses devices may experience due to large dose uniformity ratios (DURs) across packaged boxes.

Functionality tests were devised to assess detrimental effects of dose or method on device exposure and based on manufacturer guidance. Vacutainer tubes (VT) are manufactured to contain a reduced atmosphere that promotes controlled blood collection. The rubber stopper of the VT is designed to both maintain this vacuum and to provide a seal to prevent leakage and contamination after being pierced with a needle. The VT functionality tests include measurement of retained vacuum after radiation exposure and a seal leak check. The intravenous needle of the Push Button Collection Set (PB) is designed to safely retract inside its housing with activation of the button. The tubing system is designed to convey blood from the intravenous needle to the septum-piercing needle without leaking. Functional tests for the PB include confirmation of needle retraction and confirmation of no fluid leaks within the system while under pressure.

Visual appearance can give the impression of cleanliness and reliability and is a key aspect of viable medical devices. Radiation induced color changes in the components of the VT and PB devices were quantified using Yellowness Index (ASTM E313, 2015).

Hardness testing of the VT PET tubes and tensile testing of the PB PVC tubing was performed to investigate mechanical property changes in key device components with radiation exposure. The non-standard geometries of the tube and tubing specimens were tested using protocols informed by standard hardness (ASTM D785-, 2015) and tensile property (ASTM D638, 2014) methods.

The effects of dose and modality on functionality, discoloration, and mechanical properties were tested. A statistical analysis of the results determined whether the effects of e-beam or X-ray radiation processing differed from the effects of gamma radiation processing. A similar analysis confirmed the presence or absence of dose dependence in the quantities measured for each radiation method.

Details of the medical devices investigated and of the radiation exposures applied are outlined in the Materials and Methods section below, along with details of the test methods and the statistical analysis. Testing results as a function of dose and modality and a tabular summary of the outcome of the statistical analyses are provided in the Results and Analysis section. Finally, the suitability of e-beam and X-ray radiation sterilization as viable alternatives to gamma radiation sterilization for the medical devices and device components considered is discussed in the Conclusion section. The effects of radiation dose and modality on the mechanical and visual properties of injection molded specimens of key polymers from these two BD devices are described in an accompanying manuscript.

## 2. Materials and Methods

### 2.1. Medical devices

The following two medical devices, manufactured by BD, were identified as candidates for irradiation and analysis based on their widespread use and their characteristic materials:

2.1.1 *Vacutainer*<sup>TM</sup> *Plus tube (Vacutainer Tube, VT)*, BD Product number 366703. The VT, pictured in Fig. 1, is 75 mm long, 13 mm in diameter, and 3.0 mL in volume. The VT device model used was assembled without anticoagulating gel or other additive and has a paper label adhered to the tube. The commercially available version is sterilized via cobalt-60 gamma radiation. Non-sterile VT devices were graciously provided for this effort by BD.

2.1.2 *Vacutainer*<sup>TM</sup> *Push Button Blood Collection Set (Push Button, PB)*, BD Product number 367342. The PB, pictured in Fig. 2, has a 19 mm, 23-gauge proximal needle that is retractable, 305 mm tubing, a Luer adapter, and a covered distal needle. The proximal needle is designed to safely retract into the holder when the button (indicated by a small black triangle) is pushed. During use, blood flows from the intravenous needle through the tubing and into a receptacle such as a VT, necessitating the absence of leaks in the tubing and tubing/connection interfaces. PB devices are individually packaged during production. The commercially



**Fig. 1.** Shown is BD Vacutainer™ Plus tube (VT) with polymer constituents: LDPE-low-density polyethylene cap, adhesive paper label, PET-polyethylene terephthalate tube, and CIIR-chlorobutyl rubber stopper.



**Fig. 2.** Shown is BD Vacutainer™ Push Button Blood Collection Set with polymer constituents: POE-polyolefin elastomer, PPH-polypropylene homopolymer and PVC-polyvinyl chloride.

available version of the PB is sterilized via cobalt-60 gamma radiation. Non-sterile PB devices were graciously provided for this effort from BD.

## 2.2. Radiation processing

The typical dose range for ionizing radiation of 15–50 kGy is usually bioburden-based and determined according to standard methods (ANSI/AAMI/ISO, 2013, AAMI TIR17, 2017). To directly compare the effects of sterilization dose from different modalities on the VT and PB devices, batches of devices were processed to common target doses of 10, 35, 50, 80 kGy under gamma, e-beam and x-radiation. Since processing realities resulted in differences between target doses and measured doses, numerical results were extrapolated from measured dose to target dose for each modality prior to method comparison. AAMI TIR17, 2017 relates that most radiation sterilization byproducts and residuals decay or dissipate within 48 h. Due to logistical difficulties in controlling time between irradiation and testing for each of the modalities, a minimum time before test was selected as the way to control post-irradiation time variability and devices were stored out of direct light and at ambient temperature for at least 1 week following radiation processing prior to testing.

2.2.1 *Gamma* processing was performed in the research loop of a nominal 3.5 megacurie cobalt-60 BD irradiation cell in Broken Bow,

Nebraska. Devices were irradiated in boxes of trays of 100 units for the VT and smaller boxes of 50 units for the PB at 35 °C, with ozone concentrations typical for such a facility, to doses indicated in Table 1. The applied dose rate is on the order of a few Gy per second (~0.003 kGy/s).

2.2.2 *Electron beam* (e-beam) processing was performed using a 10 MeV, 15 kW s-band Varian linear accelerator at the University National Center for Electron Beam Research (ebeam-tamu.org) at Texas A&M University in College Station, Texas. Devices were irradiated individually at 22–24 °C, with ozone concentrations typical for such a facility, to doses indicated in Table 1. The VT and the PB were irradiated in their primary packaging – loaded onto the conveyor in a single layer. The associated beam current/scan settings were 1.6 mA/61 cm. The dose rate was previously empirically calculated as 3.0 thousand Gy/second. The conveyor speed was adjusted as needed to achieve the desired minimum target dose. The devices were routed through the cell multiple times, without flipping, to achieve the desired dose levels, and for a unilateral geometry. Preliminary trials were performed to understand the dose distribution in the different devices. Three independent dose mapping trials were performed, and the resulting DURs for the VT and the PB devices were 1.13 (±0.01) and 1.00, respectively.

2.2.3 *X-ray* processing was performed using a 7.5 MeV, 30 kW X-ray machine at Steri-Tek (steri-tek.com) in Fremont, California. Devices were irradiated in boxes at 21 °C, with ozone concentrations typical for such a facility, to doses indicated in Table 1. The medical devices, in trays of 100 units for the VT and boxes of 50 units for the PB, were loaded into totes on the conveyor. The associated beam scan settings were 30.5 cm from target and the conveyor speed was 0.025 m per minute. At this conveyor speed, 11 kGy was applied per pass. The speed was increased to deliver the final doses to the devices to achieve desired total doses. Resulting dose rate is on the order of a few tens of Gy per second (~0.03 kGy/s).

2.2.4 *Dosimetry* between irradiation facilities was compared to verify measured levels of absorbed dose that the samples received. Dosimetry protocols utilized were reported by each of the associated irradiation facilities. Each facility was required to provide documentation that their dosimetry system had a valid calibration traceable to the primary standard (National Institute of Standards and Technology), as well as documentation on the calculated measurement uncertainty (at the 95% confidence level) associated with their quoted product doses. The alanine dosimetry systems used at the gamma and e-beam facilities provide a measurement uncertainty of approximately 5% at the 95% confidence level, and the B3 dosimetry system used at the X-ray facility provided a measurement uncertainty of approximately 7% at the 95% confidence level. In order to provide extra assurance that the reported delivered doses at each of the three irradiation facilities were within the stated uncertainties, a dosimetry comparison study was performed that involved the TAMU e-beam facility and the Steri-Tek X-ray facility. The protocol involved Steri-Tek providing three B3 film dosimeters to TAMU, which TAMU co-located with their alanine dosimeters within an Ethafoam (closed cell polyethylene) block, then exposed the assembly to their e-beam for a targeted 12 kGy. The irradiated B3 film dosimeters were then shipped back to Steri-Tek for readout, and the alanine

**Table 1**

Target doses, average measured doses, and approximate dose rates for each modality.

|                 | 10 kGy | 35 kGy | 50 kGy | 80 kGy | Dose Rate kGy/s <sup>a</sup> |
|-----------------|--------|--------|--------|--------|------------------------------|
| Vacutainer Tube |        |        |        |        |                              |
| <b>Gamma</b>    | 10.0   | 32.5   | 51.1   | 87.5   | ~0.003                       |
| <b>E-beam</b>   | 10.7   | 38.5   | 51.6   | 71.4   | 3.0                          |
| <b>X-ray</b>    | 10.5   | 37.5   | 54.5   | 84.5   | ~0.03                        |
| Push Button     |        |        |        |        |                              |
| <b>Gamma</b>    | 10.0   | 32.5   | 51.1   | 87.5   | ~0.003                       |
| <b>E-beam</b>   | 10.9   | 35.1   | 56.5   | 73.3   | 3.0                          |
| <b>X-ray</b>    | 13.0   | 40.0   | 58.0   | 91.5   | ~0.03                        |

<sup>a</sup> Approximate values provided for order-of-magnitude comparison.

dosimeters were read out at TAMU. The resulting data is tabulated in [Table 2](#) and indicate a difference of 10% in measurement accuracy between TAMU and Steri-Tek.

### 2.3. Functional performance tests

A primary concern in considering radiation alternatives to the standard gamma sterilization method is adverse effects on device function. When defining challenge tests and acceptance criteria, the Association for the Advancement of Medical Instrumentation (AAMI) ([AAMI TIR17, 2017](#)) suggests that the tests specifically challenge the dominant or critical failure mode of the device. A second encouragement is that tests be designed to yield variable rather than attributable data. As mentioned above, key to VT performance following sterilization is retention of negative pressure in the sealed tube and resistance to liquid leaks at the stopper following needle insertion and removal. Key to PB performance following sterilization is needle retraction functionality and the absence of liquid leaks in tubing and at connections with applied pressure. Retained vacuum in the VT was quantified using a liquid draw method. Irradiated products were determined to either pass or to fail the other functional tests. Devices were randomized by dose and modality prior to testing to avoid test-order bias.

**2.3.1 Vacutainer Tube Liquid Draw (Vacuum Level) and Leak Testing.** Mass of water drawn through a collection set was used to quantitatively assess the level of vacuum in VT devices following radiation processing. Inversion of a filled VT, visual inspection and mass checking were used to determine if the stopper leaked. Six replicates of each of the four doses at all three modalities plus unirradiated samples were tested for a total of 78 samples.

The proximal end of a non-irradiated PB was fixed to the bottom of a 1 L beaker and the beaker filled with approximately 950 mL of distilled water, as shown in [Fig. 3](#). A VT to be tested was weighed using an Ohaus Adventurer™ Pro Model AV264 Analytical Balance and placed into a 120 mL jar resting on the bench next to the beaker. The distal needle of the PB was inserted into the VT through the stopper without touching the tube wall. Upon piercing the stopper, water was drawn into the VT, filling the existent vacuum. Consistent arrangement of the components assured that the hydrostatic pressure was the same for each VT tested. After the needle was withdrawn, the water-filled VT was weighed, inverted for 20 s, and weighed again to determine any leakage.

**2.3.2 Push Button Pressure and Needle Retraction Testing** The PB is designed to convey blood from the intravenous needle at one end of the device, through the connecting tubing and the septum-piercing needle at the other end of the device into a receiving container before the button is pushed and the intravenous needle retracts into the holder. A properly functioning PB will exhibit no leaks when blood is pressurized through the system and no difficulty in the needle retraction. Water was injected into each PB following radiation processing and leaks identified. Finally, the button of each PB was pressed to confirm proper needle retraction. Six replicates of each condition were tested for a total of 78 samples.

The PB pressure test apparatus is shown in the [Fig. 4](#). It consists of a 1 L beaker, a Fluke Model 179 True-RMS Digital Multimeter with Fluke Model PV350 Pressure Vacuum Module, a 20 mL syringe, and a rubber stopper, T-fittings and clamps. Water was dyed (Denim Blue, Rit Dye) to aid with visibility. Each PB device was connected to the test apparatus

**Table 2**

Results of dosimetry comparison between E-beam and X-Ray irradiation facilities.

| Dosimeter #       | TAMU Alanine Readings (kGy) | Steri-Tek B3 Readings (kGy) |
|-------------------|-----------------------------|-----------------------------|
| 1                 | 13.9                        | 12.5                        |
| 2                 | 13.7                        | 12.2                        |
| 3                 | 13.4                        | 12.2                        |
| Mean:             | 13.6                        | 12.3                        |
| Difference: < 10% |                             |                             |

and colored water was drawn into the device with the syringe. The proximal needle was then inserted into the stopper, and pressure applied to the device system. Using the connected pressure sensor for verification, a pressure of at least 100 kN/m<sup>2</sup> was applied for at least 20 s. Presence of any leaked water was then determined. To verify needle retraction, the convex part of the device located just below the winged tabs was held during activation of the button. If the needle fully retracted upon button press, the device was deemed to pass the test.

### 2.4. Device discoloration

Undesirable color change, such as yellowing, can occur in medical devices because of sterilization processing. Yellowness Index (YI) ([ASTM E313, 2015](#)) was measured for select materials in the devices considered for each dose and modality: the PET tube, paper label, LDPE cap, and CIIR stopper for the Vacutainer Tube; and the POE wings, PPH finger grip, and PVC tubing for the Push Button. A Nikon D5600 camera with Nikon 100 mm f/2.8 Series E AIS manual focus lens and a Color Assessment Cabinet were used to photograph each device along with an X-Rite ColorChecker Classica White Board (X-Rite, Inc.). Nikon Electronic Format (NEF) raw files were converted to Digital Negative (DNG) format using Adobe Digital Negative Converter software. ColorChecker Passport software was used to create a profile specific to the camera used and Adobe Photoshop software was used to apply the camera profile to the photos. MATLAB® was used to calculate the YI and RGB (Red Green Blue) values at the 95% confidence interval. Six devices for each irradiation condition were analyzed for a total of 78 devices.

### 2.5. Mechanical testing of select components

Standard mechanical tests such as tensile strength ([ASTM D638-14, 2014](#)) and hardness ([ASTM D2240-15, 2015](#)) may be used to compare the effects of radiation dose and modality on plastics in medical devices to be sterilized. These tests prescribe specific shapes and dimensions for testing to account for the influence of specimen geometry on material performance that are impractical to obtain from manufactured devices. The effects of gamma, e-beam and X-radiation on injection molded standard geometry test specimens of device polymers are considered in an accompanying manuscript. Here the relative hardness and tensile properties were measured using ASTM-inspired methods, but on device components in their as-produced geometries.

**2.5.1 VT PET Tube Hardness Testing.** A Rex Model DD-5-D Type D Digital Durometer with a Rex Model OS-1 Operating Stand was used to obtain hardness values for the clear PET tube of the VT device as seen in the [Fig. 5](#). A quantity of 15 unirradiated devices and 20 irradiated samples (5 replicate samples from each of the 4 doses) from each method (gamma, electron-beam, and x-ray) were prepared, giving a total of 75 test samples for Shore D hardness testing of the PET tubes. Devices were randomized by dose and modality prior to testing to avoid test-order bias. For testing, each sample was placed in a custom designed holder/slider to ensure that the sample did not move during the test. Seven locations on the tube holder were marked for aligning to a mark on the slider, to guide positioning of the indenter above the desired testing location of the tube. The points of interest on the tube were placed 6 mm apart ([ASTM D2240, 2015](#)) with the first point being 12 mm from the curved base of the tube. The tube holder and slider were designed to ensure that the indenter contacts the highest vertical location of the tube as it lies on its side. The operator slowly lowered the handle to move the presser foot flush with the sample surface, as specified in Section 9.2.3 of ASTM D2240. The maximum hardness value was recorded for each of the seven measurement locations.

**2.5.2 Push Button PVC Tubing Hardness Testing.** A Rex Model DD-4 Type M Digital Durometer with a pneumatically damped Rex Model OS-3 Operating Stand was used to obtain hardness values for the flexible tubing of the PB device as seen in the [Fig. 6](#). A quantity of 5 unirradiated samples and 20 irradiated samples (5 replicate samples from each of the



Fig. 3. VT liquid draw test setup (Left). VT inverted leak check (Right).



Fig. 4. Assembled test component for the Push Button Pressure test (Left), liquid filling (Center), and pressure application (Right).



Fig. 5. Shore D durometer testing apparatus, showing testing of PET in Vacutainer Tube.

4 doses) from each method (gamma, e-beam, and X-ray) were tested. This yielded a total of 65 test samples for Shore M hardness testing of the PB device PVC tubing. Tubing specimen dimensions were 75 mm length, 2 mm tube outer diameter, and 1.1 mm tube inner diameter. Seven measurement locations were marked 6 mm apart (ASTM D2240, 2015) using a permanent marker, with the outer points being roughly 20 mm from the end of each specimen. A custom fixture was used to hold the tubing specimens in place during testing. Specimens were laid in a 2 mm diameter groove to align the tubing center with the durometer indenter tip. Two steel blocks held the specimen flat in the groove. Fine-tip tweezers were used to pull the specimen until the indenter tip aligned

with each measurement location. The weight of the steel blocks applied a consistent tension to the specimen during each sliding motion. For each measurement the durometer was released from maximum height and allowed to contact the specimen at a smooth rate of descent controlled by the pneumatically damped operating stand. The maximum hardness value was recorded for each of the 7 measurement locations.

2.5.3 *Push Button PVC Tubing Tensile Testing.* An Instron 5943 Universal Testing System with 1 kN load cell was used to measure force during controlled extension of the PVC tubing specimens. Video extensometry was performed using a digital camera and a custom-written video analysis method. Sets of 16 unirradiated control



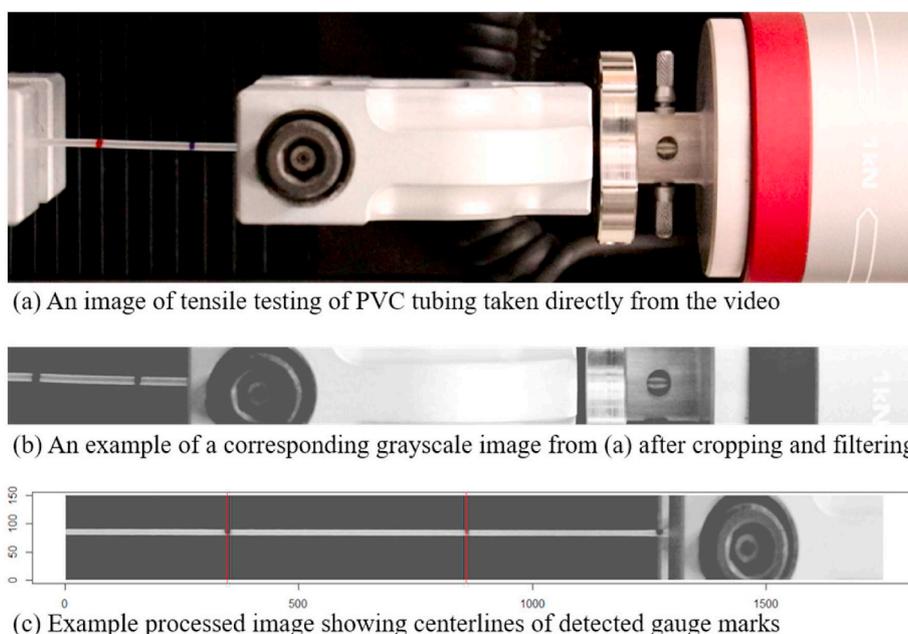
**Fig. 6.** Shore M durometer with custom fixture to hold PVC tubing specimens centered under the durometer indenter tip. Two steel blocks hold the specimen flat in the fixture groove.

specimens and 24 irradiated specimens (6 replicate samples from each of the 4 doses) from each method (gamma, electron-beam, and X-ray) were prepared, giving a total of 88 test specimens. Specimens were randomized by dose and modality prior to testing to avoid test-order bias. The dimensions of the specimens were 115 mm total length, 65 mm between grips, 25 mm gauge length, 2 mm tube outer diameter, and 1.1 mm tube inner diameter. The gauge length marks were indicated on each specimen using a fine-tipped permanent marker for visibility use in video extensometry. All tensile tests were performed at 22 °C at a displacement rate of 50 mm/min. The video extensometry package used MATLAB® and R programs (R Core Team, 2019) to determine strain data for each tensile test. A digital single-lens reflex camera recorded videos of each specimen during tensile testing, focusing on the gauge marks. The gauge marks were drawn at the top and bottom of the gauge section, oriented across the specimen width perpendicular to the test direction. MATLAB® was used to extract frames from the videos, crop and convert

them to grayscale, apply a threshold to remove visible background features, and apply a 3 by 3 median filter to reduce noise while preserving edge boundaries. An R program routine determined gauge mark locations by searching for high-contrast regions at the gauge mark edges. Strain was calculated using pixel distances between gauge marks. The time at break was automatically determined and subsequently used by a second MATLAB® routine to align and combine the video extensometry strain data with the stress data recorded by the tensile test frame. Fig. 7 shows examples of images used to calculate strain values with the video extensometry package.

## 2.6. Statistical analysis

Gamma radiation is currently used to sterilize the 5 billion VTs and 0.25 billion PBs manufactured each year. This work seeks to understand how sterilization effects from e-beam or X-ray might differ from the



**Fig. 7.** Example images of a PVC tubing specimen during tensile testing used to calculate the strain values from video extensometry via an image processing program.

effects of gamma sterilization. Statistical analysis targeted comparison of tested properties for 1) e-beam versus gamma radiation and 2) X-ray versus gamma radiation at the dosage levels investigated. Analysis was performed using R software (R Core Team, 2019) and based on a significance level of 0.05. Response variables for the analyses were the recorded values for the tests performed. The factor variables were the modality and irradiation dose. Radiation method had 3 levels – gamma, e-beam, and X-ray. Dose had four target levels – 10, 35, 50, and 80 kGy, but actual processing doses were used for many of the statistical analyses. For each property, the analytical objectives were to compare property values by modality and dose and to identify cases where property values differ significantly due to these two factor variables, and in particular, where property values for e-beam differ significantly from gamma radiation or values from X-ray differ significantly from gamma radiation.

T-tests for two independent samples were conducted and considered as primary tests for this work because they most directly compare property values from e-beam versus gamma radiation or X-ray versus gamma radiation modalities. Equal variance and unequal variance versions of the T-tests were conducted. Additional parametric tests were also conducted that look for overall property differences among the three irradiation modalities at the different irradiation dosage levels. The additional parametric tests conducted were one-way ANOVA F-test (Neter, 1990), Tukey's HSD (honestly significant difference) test, Dunnett's test (Dunnett, 1955) with Control cases (non-irradiated specimens) as the control, and Dunnett's test with gamma radiation specimen as the control.

The parametric tests used for this work were typical normal distribution-based methods that require normally distributed data within treatments and a common variance across the different treatments (homogeneity of variance). To check the assumptions associated with parametric tests the Kolmogorov-Smirnov, Anderson-Darling, and Shapiro-Wilkes tests were used to check the normality assumptions; Bartlett's test and Hartley's test were used to check the homogeneity of variance assumptions.

Along with these parametric tests, two non-parametric tests (Daniel, 1990) were also conducted. The non-parametric methods do not require normally distributed data but do assume the distributions have the same shape for the different treatments (so essentially the same variance). The non-parametric tests conducted were the Kruskal-Wallis (K-W) test and the median test. When the necessary assumptions are satisfied, the parametric tests are preferred as they are generally considered to be more powerful than non-parametric tests. For cases where the assumptions are not satisfied, the non-parametric tests are preferred. These same preferences were applied for this work when determining a conclusion for a particular test scenario.

The six statistical tests described above (the equal variance T-test, the unequal variance T-test, Tukey's HSD test, Dunnett's test, the K-W test, and the median test) were used to compare the quantitative result of the characterization of an e-beam or X-ray irradiated sample to that of a gamma irradiated sample for each dose. If at least 5 of the 6 indicated no divergence between the gamma irradiated sample results and the e-beam or X-ray irradiated sample results with 95% confidence for that dose, then a "not significant" result was drawn. If at least 5 of the 6 tests indicated a diversion between the value from the alternative modality and the value from gamma then a statistically "significant" result was drawn. If fewer than 5 of the 6 tests converged on the same conclusion then the result was deemed "inconclusive". For each dose, the differences between the results of quantitative tests for each of the two alternative modalities and gamma are summarized as statistically significant (S), statistically not significant (NS), or statistically inconclusive (I) in Table 3.

The VT leak, PB leak, and PB needle retraction functional tests were Pass/Fail in nature. That is, either the device performed the intended function properly or it did not. Using the Pass/Fail data, a success rate can be calculated for each test and a 95% lower confidence limit on the success rate estimated for each test. These lower confidence limits provide a statistical statement that with 95% confidence the success rate for a device and function is equal to or greater than the calculated value. For example, if for a device and function, 30 out of 30 functionality tests

**Table 3**

Statistical equivalency comparison of e-beam and X-ray results to gamma results at each irradiation dose level. Orange indicates significant (S) differences; green indicates no significant (NS) differences; and no color fill indicates inconclusive (I) determination of significant differences.

| Equivalency to Gamma for Quantitative Tests   | 10 kGy |       | 35 kGy |       | 50 kGy |       | 80 kGy |       |
|---|--------|-------|--------|-------|--------|-------|--------|-------|
|   | E-beam | X-ray | E-beam | X-ray | E-beam | X-ray | E-beam | X-ray |
| <b>Vacutainer Tube</b>  |        |       |        |       |        |       |        |       |
| Liquid Draw (Vacuum Level)  | S      | S     | NS     | S     | NS     | S     | S      | S     |
| PET Tube – Yellowness Index   | S      | NS    | S      | NS    | NS     | NS    | NS     | NS    |
| LDPE Cap – Yellowness Index   | NS     | NS    | NS     | NS    | NS     | NS    | NS     | NS    |
| Paper Label – Yellowness Index  | NS     | NS    | NS     | NS    | NS     | NS    | NS     | NS    |
| CIIR Stopper – Yellowness Index   | NS     | NS    | NS     | NS    | NS     | NS    | NS     | NS    |
| PET Tube – Hardness   | NS     | NS    | NS     | NS    | NS     | I     | NS     | NS    |
| <b>Push Button</b>  |        |       |        |       |        |       |        |       |
| POE Wing – Yellowness Index   | S      | NS    | NS     | NS    | NS     | NS    | S      | S     |
| PPH Grip – Yellowness Index   | NS     | NS    | I      | NS    | S      | NS    | NS     | S     |
| PVC Tubing – Yellowness Index   | NS     | NS    | NS     | NS    | NS     | NS    | S      | S     |
| PVC Tubing – Tensile Strength   | NS     | NS    | NS     | NS    | NS     | NS    | NS     | NS    |
| PVC Tubing – 10% Secant Modulus   | NS     | NS    | NS     | NS    | NS     | NS    | NS     | NS    |
| PVC Tubing – Elongation at break  | NS     | NS    | NS     | NS    | NS     | NS    | NS     | NS    |
| PVC Tubing – Shore M Hardness   | S      | NS    | NS     | NS    | NS     | NS    | NS     | NS    |
| NS– Difference <i>Not Significant</i> , S–Differences <i>Significant</i> , I–Inconclusive |        |       |        |       |        |       |        |       |

NS– Difference *Not Significant*, S–Differences *Significant*, I–Inconclusive.

resulted in success, then the 95% lower confidence limit would be calculated to be 0.905. Thus, with 95% confidence, the success rate for that device would be at least 90.5%. The 95% lower confidence limit on the success rate was calculated using the [Clopper-Pearson](#) method. This involves the binomial probability distribution.

### 3. Results and Analysis

#### 3.1. Vacutainer Tube

**3.1.1 VT Liquid Draw (Vacuum Level) Test.** Results of the liquid draw test for the VT are provided below in [Fig. 8](#), which plots water draw values as a function of dose for each modality. Bounding levels labeled with percent fraction of average unirradiated sample result are included in the plot to reflect relative magnitude of variation in liquid draw following irradiation. Results indicate that the 10 kGy dose level slightly increases the vacuum level inside the VT compared to the unirradiated VT and that the vacuum level is equivalent for a given modality at all doses considered above 10 kGy. The mechanism for increase in vacuum level with the VT following X-irradiation is not clear. Perhaps radiation-induced oxidation of polymer in the interior of the VT consumes oxygen trapped in the device thus lowering the number of moles of gas in the volume of the VT. Statistical analyses determining equivalency between alternative modalities and gamma radiation as a function of dose for quantitative tests are summarized in [Table 3](#). Analyses indicates a statistical difference between both the results of both e-beam and X-radiation and the results of gamma irradiation at almost all doses. Vacuum level appeared to increase within the VT for all irradiated devices relative to the unirradiated control.

**3.1.2 Vacutainer Tube Stopper Leak Test.** None of the 88 VT devices tested were observed to leak when inverted after water fill. Using the [Clopper-Pearson method](#), based on the absence of failure in 72 of 72 irradiated devices, the success rate for similarly irradiated devices is calculated to be at least 96% with 95% confidence.

**3.1.3 Vacutainer Discoloration.** The following figures display discoloration results for the four components of the VT versus both dose level and modality: PET tube, paper label, LDPE cap, and CIIR stopper. Location of color analysis on the device is indicated in the inset image in each plot. In [Fig. 9](#), the PET tube shows a clear increase in YI with increasing dose for all three modalities. [Fig. 10](#) shows an apparent linear increase in YI for the paper label of the VT for all three modalities. The increase in YI with dose for the LDPE cap in [Fig. 11](#) is less pronounced, and no apparent trend is observed for the YI of the CIIR stopper as seen

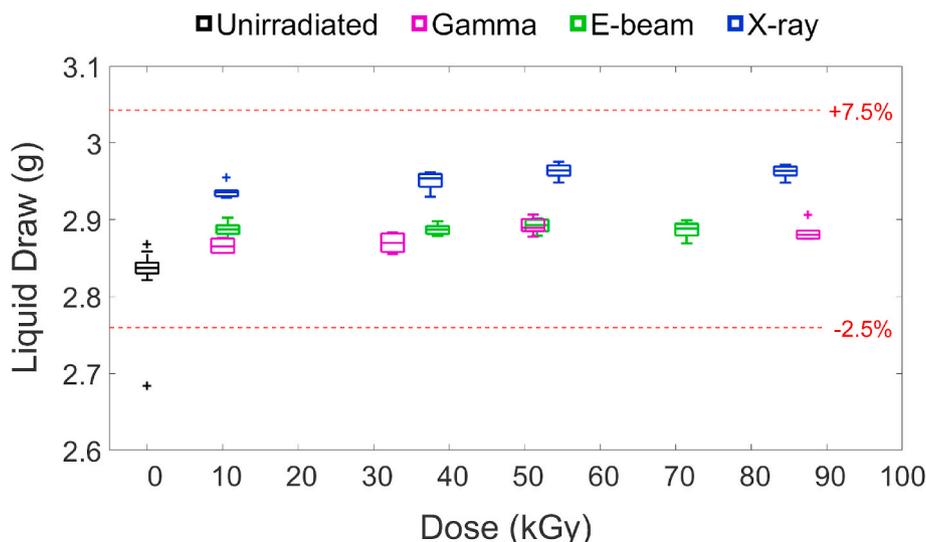
in [Fig. 12](#). Relative magnitude of variation in tested values following irradiation are indicated in the plots by labeled bounding levels showing percent fraction of average unirradiated sample result. [Table 3](#) summarizes the analysis indicating that changes in YI with e-beam irradiation were statistically different than those of gamma irradiation for the PET tube at the 10 and 35 kGy lower doses explored. Changes in YI with e-beam and X-ray irradiation were found to be statistically equivalent to YI changes for gamma irradiation for all other materials and doses.

**3.1.4 Vacutainer PET Tube Hardness.** The results of Shore D hardness measurements on the PET tubes of irradiated VT devices versus dose shown in [Fig. 13](#) and [Table 3](#) indicate no statistically significant effect of irradiation or modality on hardness for the doses explored. Hardness of the PET tubes thus appears to be insensitive to irradiation dose and up to ~80 kGy for the three modalities. Relative magnitude of variation in hardness values following irradiation is indicated in the plot by bounding levels showing percent fraction of average unirradiated sample result.

#### 3.2. Push Button Blood Collection Set

**3.2.1 Push Button Pressure and Needle Retraction Testing.** Of the 72 irradiated PB devices tested at pressure, none were observed to leak. Likewise, the needles retracted as designed in all 72 of the irradiated PB devices tested. Thus, the leak and retraction success rates for similarly irradiated devices are calculated to be at least 96% with 95% confidence.

**3.2.2 Push Button Discoloration.** The following figures chronicle discoloration results for the three components of the BT versus both dose level and modality: POE wings, PPH finger grip, and PVC tubing. Location of color analysis on device is indicated in the inset image in each plot. The results generally reveal a dose dependence on discoloration, but no difference between modalities. Overall changes in YI for the POE wings is small, as seen in [Fig. 14](#), perhaps due to the blue color of this POE formulation. Nonetheless, YI of the e-beam irradiated POE is statistically different than YI for the gamma irradiated POE at 10 kGy. At 80 kGy, the YI of both the e-beam and the X-irradiated POE wings was found to differ from the YI of gamma-irradiated POE wings. YI exhibited a much greater change with dose for the PPH finger grips for all modalities as seen in [Fig. 15](#). The change in YI of e-beam irradiated PPH at 50 kGy and X-irradiated PPH at 80 kGy were statistically different than the change in YI of gamma irradiated PPH at the corresponding doses. The change in YI of PVC tubing is seen in [Fig. 16](#) with processing to exhibit an intermediate dose dependence. YI change at 80 kGy was



**Fig. 8.** VT Liquid draw versus dose for all three modalities.

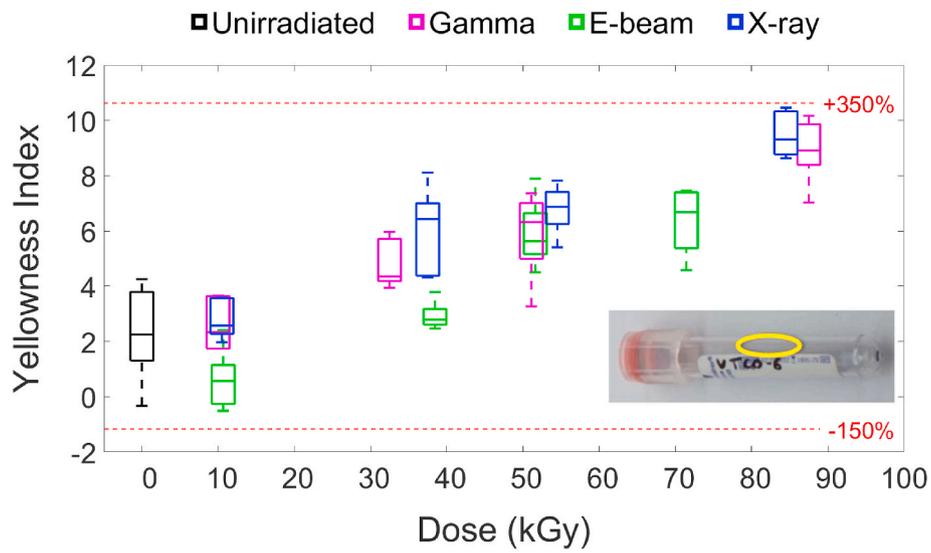


Fig. 9. Yellowness Index of VT PET tube versus dose for all three modalities.

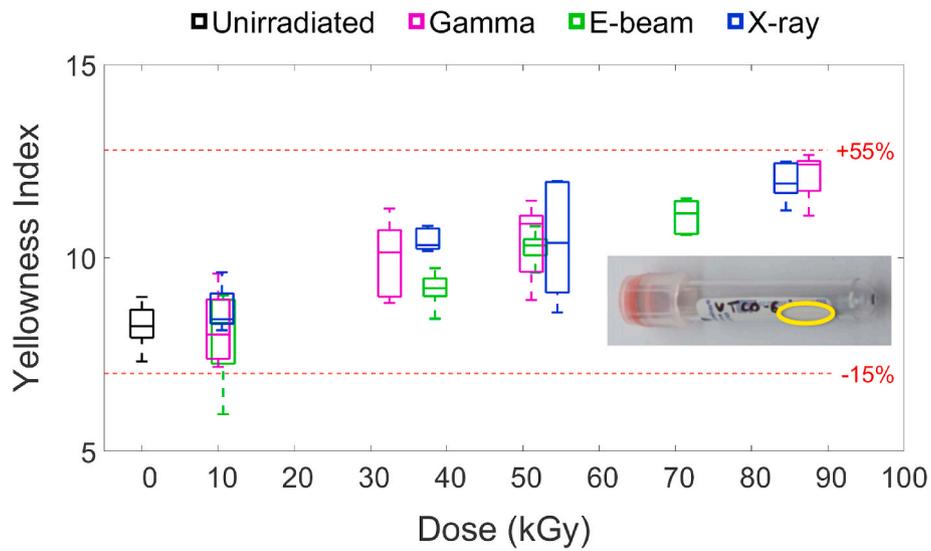


Fig. 10. Yellowness Index of VT paper label versus dose for all three modalities.

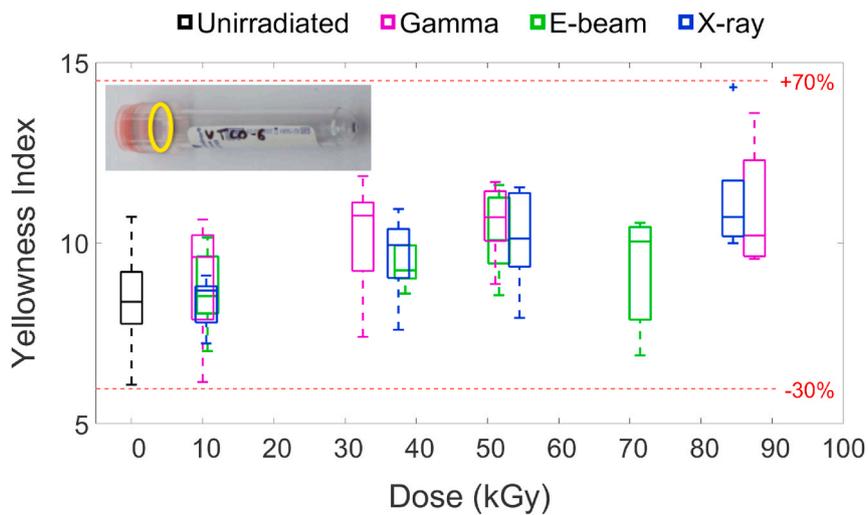


Fig. 11. Yellowness Index of VT LDPE cap versus dose for all three modalities.

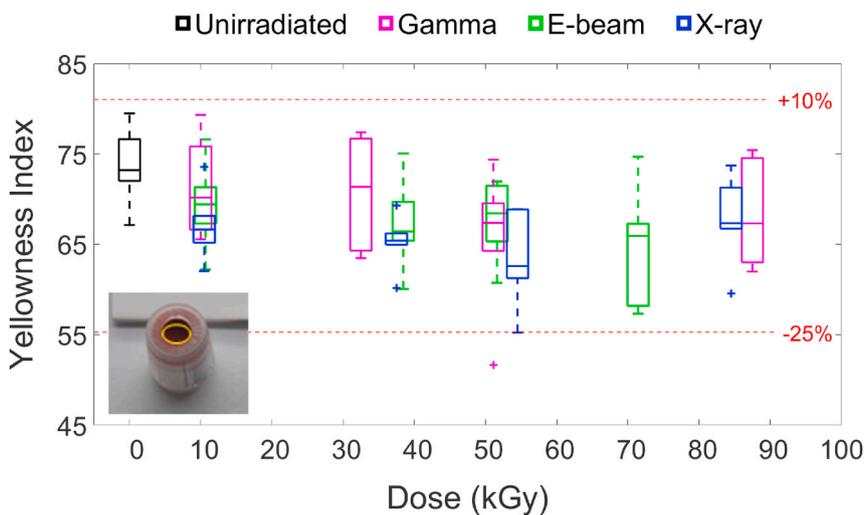


Fig. 12. Yellowness Index of VT CIIR stopper versus dose for all three modalities.

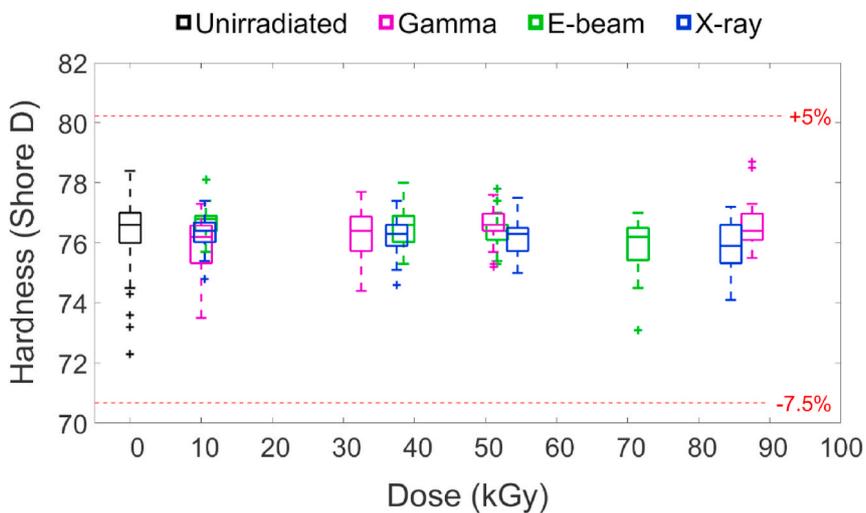


Fig. 13. VT PET tube Shore D hardness versus dose for all three modalities.

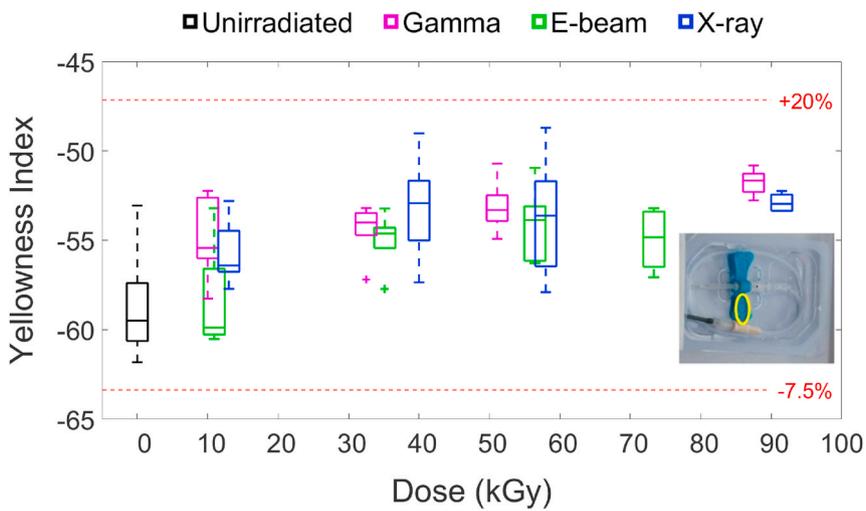


Fig. 14. Yellowness Index of PB POE wings versus dose for all three modalities.

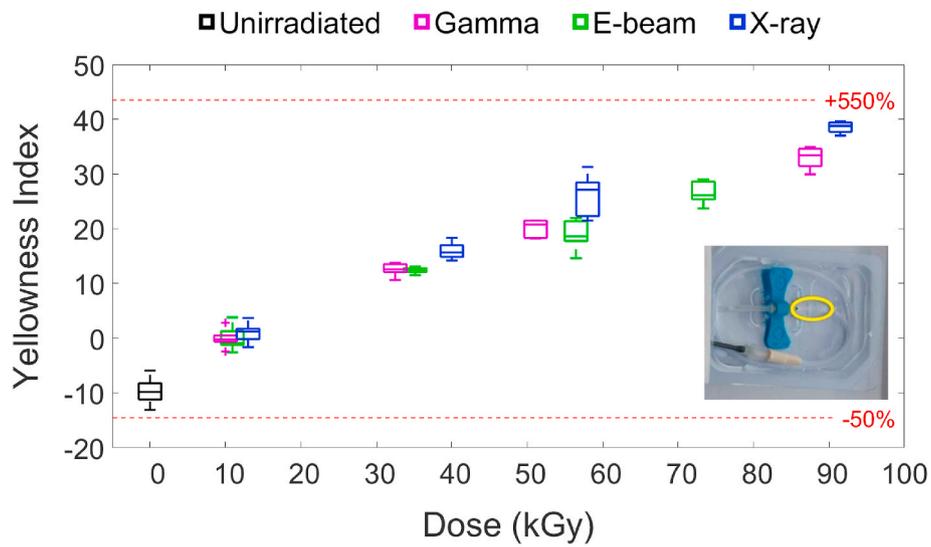


Fig. 15. Yellowness Index of PB PPH stopper versus dose for all three modalities.

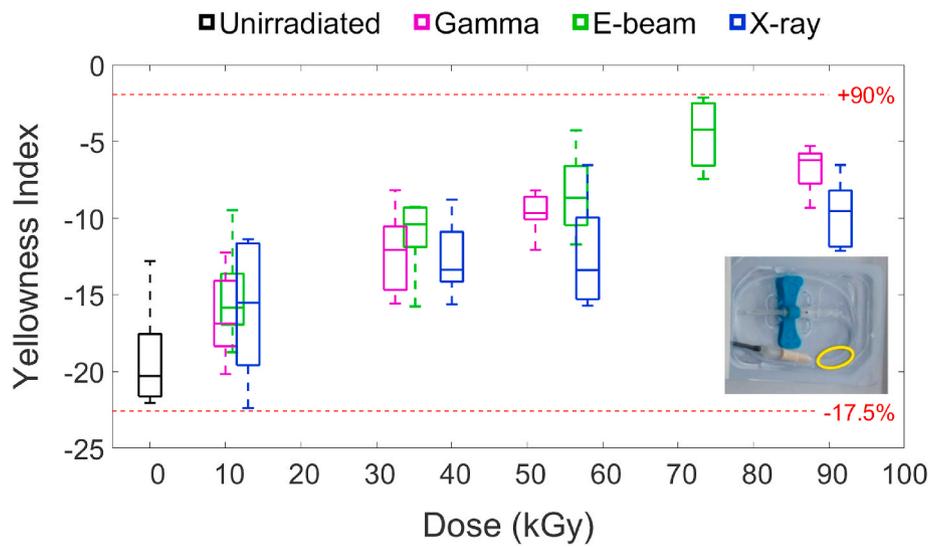


Fig. 16. Yellowness Index of PB PVC tubing versus dose for all three modalities.

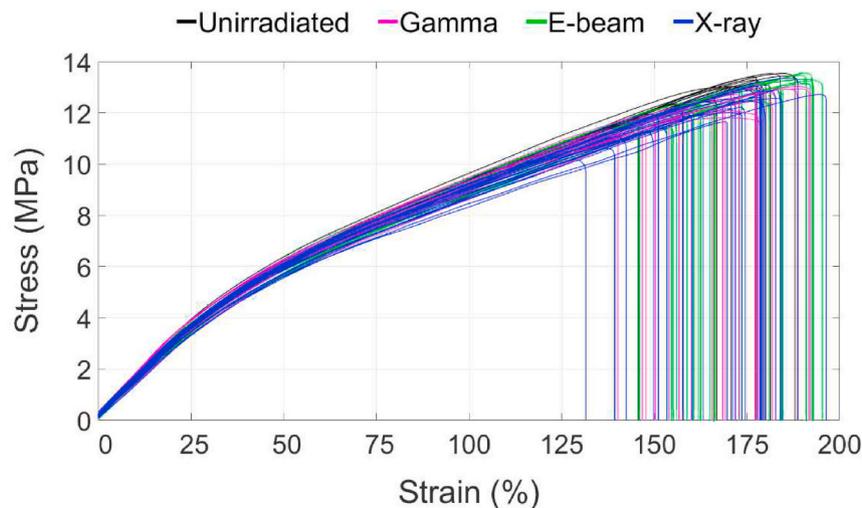


Fig. 17. Stress-strain curves from tensile testing of Push Button PVC tubing.

observed to differ from that of gamma irradiated PVC for both e-beam and X-irradiated tubing. Relative magnitude of variation in YI values following irradiation is indicated in the plots by labeled bounding levels showing percent fraction of average unirradiated sample result. Statistical analysis results of irradiation differences versus dose for the three PB materials considered are summarized in Table 3.

**3.2.3 Push Button PVC Tubing Tensile Testing.** Tensile test stress-strain curves of PB PVC tubing generated with extensometry are plotted in Fig. 17. PVC tubing strength, 10% secant modulus, and elongation at break, plotted in Figs. 18–20, respectively, demonstrated no dose dependence. The medians of each test set seem to indicate a slight decrease in PVC tubing strength with exposure but were within the variation of the measurement and of no statistical significance. Statistical analysis of effects on tensile properties, summarized in Table 3, showed no difference between modalities. Relative magnitude of variation in tensile values following irradiation is indicated in the plots by labeled bounding levels showing percent fraction of average unirradiated sample result. The elongation at break in Fig. 20 indicates a moderate level of scatter for at each dose and modality that likely stems from testing artifacts of the uniform cross-section tubing specimens (some specimens broke near the grips). Overall, the tensile properties of the PVC tubing from the PB devices appears to be insensitive to dose and modality up to the ~80 kGy investigated.

**3.3.4 Push Button PVC Tubing Hardness.** The Shore M hardness results for PVC tubing specimens from the PB devices versus dose for each modality are shown in Fig. 21. Relative magnitude of variation in hardness following irradiation is indicated in the plot by bounding levels showing percent fraction of average unirradiated sample result. The data do not seem to indicate a dose dependence for hardness. There is a statistical difference between e-beam irradiation effects and gamma irradiation effects on PVC tubing hardness for 10 kGy dose as indicated in Table 3. The hardness of PB PVC tubing appears to be insensitive to radiation dose and method up to ~80 kGy.

#### 4. Conclusions

The U.S. Food and Drug Administration requires testing be performed on new medical devices requiring sterility and on legacy medical devices that a manufacturer desires to switch to another sterilization method to ensure safety (functionality, biocompatibility, and ability to sterilize). ISO 11137-1 guidance provides expectation of being able to transfer maximum acceptable dose from a low dose rate gamma sterilization process to a higher dose rate e-beam or x-ray process. AAMI

Technical Information Report (TIR) 17 (AAMI, 2018) “*Compatibility of Materials Subject to Sterilization*” provides guidance for such testing. In this work, the effects of e-beam and X-ray radiation modalities on two high volume medical devices were directly compared to the effects of the standard cobalt-60 gamma radiation to identify any differences. Relevant functional performance tests and ASTM-type mechanical tests as recommended in AAMI TIR17 were followed in the study. Rigorous statistical analyses were performed on the data to determine significant differences in polymer effects between those devices processed with gamma radiation and those processed with e-beam or X-ray radiation.

Functionality, discoloration and mechanical characteristic changes with exposure to sterilization relevant doses from three different modalities were assessed for two major BD medical devices – the *BD Vacutainer™ Plus tube*, and the *BD Vacutainer™ Push Button Blood Collection Set*. Functional performance tests were performed using intact final devices to simulate the physical forces and movements that these devices undergo when used by the end users (healthcare personnel and patients). For the VT, tests included liquid draw and leak testing to assess intact vacuum and seal integrity. For the PB, tests included liquid system integrity and effective needle retraction. These tests were performed on approximately 250 devices and included irradiation by cobalt-60 gamma-ray, e-beam and X-ray methods to four different dose levels ranging from 10 to 80 kGy.

VT seal integrity, PB liquid system integrity and PB needle retraction were applied as Pass/Fail tests. None of the devices failed the tests for the modalities and dose levels investigated. For the liquid draw assessment of VT vacuum level following irradiation, the X-ray irradiated devices exhibited improvement in performance as demonstrated by increase in water draw following irradiation. Neither e-beam nor X-ray processing lowered functional performance of the devices at sterilization-relevant doses compared to gamma processing for the tests performed.

Effect of irradiation and method on hardness was assessed for the PET tube of the VT and both hardness and tensile properties for the PVC tubing of the PB. The results of these mechanical tests indicated no statistical difference between modalities or dose levels over the conditions explored. Neither e-beam nor X-ray processing lowered the mechanical properties of the devices at sterilization-relevant doses compared to gamma processing for the tests performed on the components tested.

While discoloration may not affect the safety of sterilized medical devices, appearance is important in communicating cleanliness, quality and consistency to consumers. Significant changes in coloration

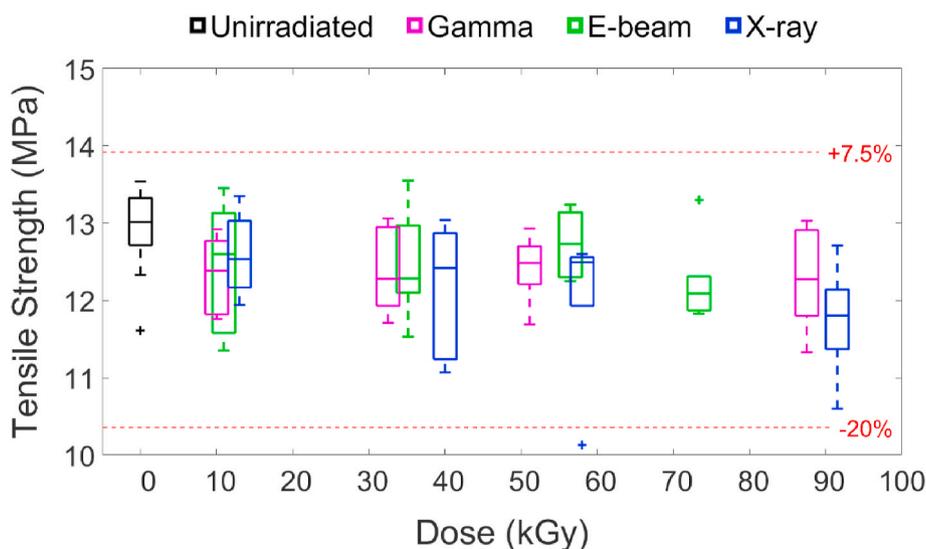


Fig. 18. Push Button PVC tubing ultimate tensile strength versus dose.

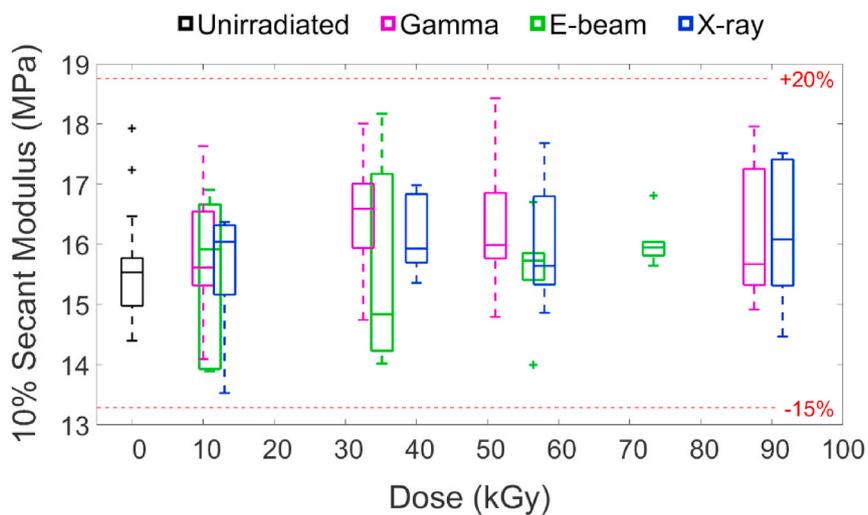


Fig. 19. Push Button PVC tubing 10% secant modulus versus dose.

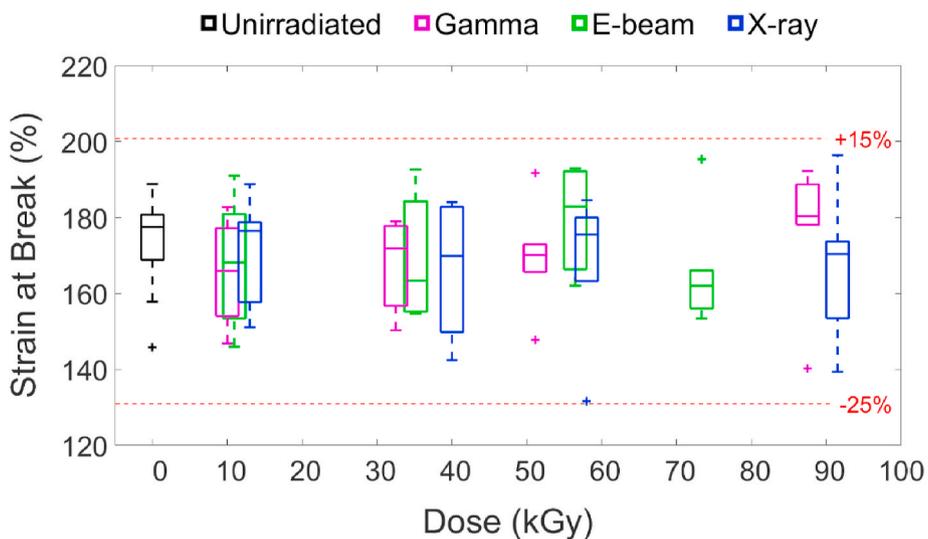


Fig. 20. Push Button PVC tubing elongation at break versus dose.

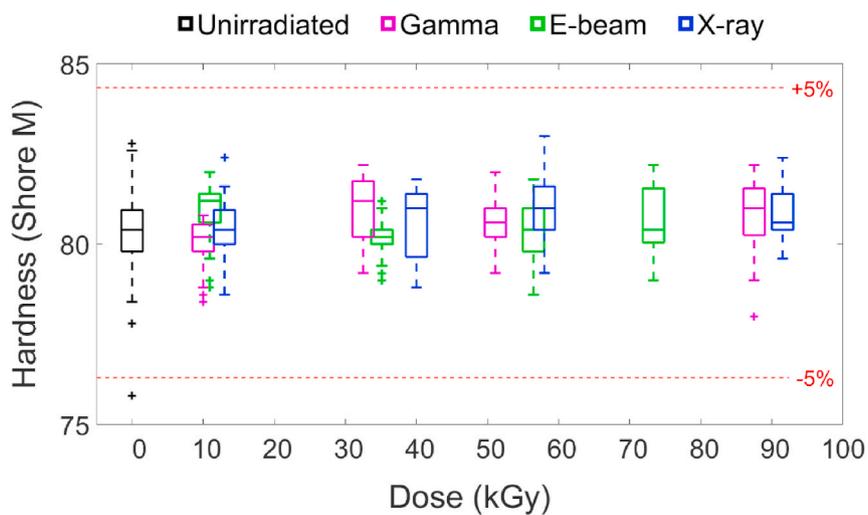


Fig. 21. Push Button PVC tubing Shore M hardness versus dose.

observed by long-time users of a device following transition to an alternative sterilization method could result in questioning of the quality or safety of the device. For this reason, it may be desirable that a change in sterilization method not result in significant color change from the baseline method. Statistical differences between the effects of e-beam and X-ray processing and gamma processing on discoloration of the PET, LDPE and CIIR components of the VT, as measured by changes in YI, were only identified for e-beam at the lower doses of 10 and 35 kGy for the PET tube. In those cases, the PET yellowed less following e-beam processing than with gamma processing. For the PB components, the results were more mixed. Change in YI of the POE wing following processing was found to be statistically different between e-beam and gamma at 10 kGy and between both e-beam and X-ray versus gamma at 80 kGy. Degree of discoloration of the blue POE wing, however, was small for all variants tested. X-ray processing was found to be statistically different than gamma processing on the color change of the PPH finger grip at 80 kGy and e-beam processing for the PPH finger grip at 50 kGy. Both e-beam and X-ray processing resulted in color changes that were statistically different than gamma processing of PVC tubing at 80 kGy.

No testing or characterization of the BD products in this work was performed for at least one week following irradiation to allow potential sterilization byproducts and residuals to decay or dissipate. The testing reported was completed within approximately six to nine months following irradiation. The dose and modality dependence of the effects of irradiation observed were assessed with the assumption of no dependence of the results on time since irradiation. Considering that storage conditions are mild compared to irradiation conditions it is expected that the observed device and material performance as a function of modality is determined by the radiation processing itself and the results are therefore representative of performance throughout product shelf life. Manufacturers may require additional data directly comparing irradiation modality effects with regard to device shelf life, which could be a focus of future work.

The results described here support the expectation from the guidance in ISO 11137-1 that electron-beam and X-ray methods are viable alternatives to cobalt-60 gamma radiation sterilization of the nearly 5.3 billion devices produced by BD each year represented by the two investigated here. These data on devices made from PET, LDPE, CIIR, POE, PPH, and PVC may encourage other medical device manufacturers to consider e-beam and X-ray as potential sterilization alternatives to gamma radiation for devices fabricated from similar polymers. Injection molded specimens of key polymers from these two BD devices were produced for direct evaluation of the effects of modality and dose levels on polymer mechanical and visual properties. That study and the observed results are described in an accompanying manuscript.

The direct comparison of effects of the three irradiation modalities of this work is intended to give confidence to the industry regarding transition from gamma to e-beam or X-ray. The testing described may not be representative of the scope of testing required for all conversion programs. Per guidance in ISO 11137-1, however, it is anticipated that an understanding of the theoretical physics underpinning the three modalities and rigorous examples such as this work will help to

minimize qualification requirements in modality transition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.radphyschem.2020.109282>.

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