

Table 1. Details of methods used by participating laboratories.

	Laboratory 1	Laboratory 2	Laboratory 3
RNA isolation method	PAXgene Blood RNA Kit (Qiagen)	PAXgene Blood RNA kit (Qiagen)	PAXgene Blood RNA Kit (Qiagen)
cDNA synthesis method	High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific)	High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific)	High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific)
qPCR kit/mastermix	TaqMan Universal Master Mix II, no UNG (Thermo Fisher Scientific)	PerfeCTa Multiplex qPCR SuperMix (Quanta Biosciences Inc. Gaithersburg, MD, USA)	TaqMan Universal Master Mix (Thermo Fisher Scientific)
Primers and probes for FDXR	TaqMan assay Hs00244586_m1	Primer F: GTACAACGGGCTTCCTGA GA Primer R: CTCAGGTGGGGTCAGTAG GA Probe: CGGGCCACGTCCAGAGCC A	TaqMan assay Hs01031617_m1
Primers and probes for reference gene	TaqMan assay Hs00229263_m1 (ITFG1)	(HPRT1) Primer F: TCAGGCAGTATAATCCAA AGATGGT Primer R: AGTCTGGCTTATATCCAA CACTTCG Probe: CGCAAGCTTGCTGGTGAA AAGGACCC	TaqMan assay Hs99999901_s1 (18S rRNA)
PCR conditions	1x/95°C/10min, 40x/95°C/15s, 60°C/1min	1x/95°C/2min, 45x/95°C/10s, 60°C/1min	1x/50°C/2min, 1x/95°C/10min, 40x/95°C/15s, 60°C/1min
Detection system	7500 Real-Time PCR System (Thermo Fisher Scientific)	Rotor-Gene Q (Qiagen)	QuantStudio 12K OA Real-Time PCR System (ThermoFisher Scientific)

Table 2. Dose estimates of blind samples calculated from FDXR expression data generated independently by three laboratories.

Sample	Estimated dose (Gy)			True dose (Gy)
	Lab 1	Lab 2	Lab 3*	
A	2.011	1.737	1.198	1.800
B	0.024	0.020	0.023	0.000
C	0.381	0.369	0.373	0.400

* In the case of Lab 3, the calibration curve without 2 Gy dose point was used to calculate dose estimates (see Figure 1 data).

Table 3. Dose estimates of blind samples based on laboratory 1 data and a linear calibration curves presented in Figure 2 for which the non-irradiated sample was excluded or represented as 0.01 Gy or 0.001 Gy.

Sample	Estimated dose (Gy)			True dose (Gy)
	Curve without 0 Gy	Curve with 0 Gy as 0.01 Gy	Curve with 0 Gy as 0.001 Gy	
A	2.025	2.149	2.383	1.800
B	0.027	0.010	0.002	0.000
C	0.401	0.287	0.160	0.400

Table 4. Summary of the available studies on FDXR expression in blood after doses ≤ 50 mGy.

Model	<i>ex vivo/in vivo</i>	Dose	Radiation type	Time points	Method	Main Findings	Reference
Cardiac fluoroscopy patients and CT patients.	<i>in vivo</i>	9-76 mSv (Cardiac Fluoroscopy Patients); 2.9-20.9 mGy (CT Patients)	X-rays	24 h, 1 month (Cardiac Fluoroscopy Patients); 2 h, 6h, 24 h, 2 d, 3d, 7d (CT Patients)	qPCR	FDXR expression appeared generally unchanged for cardiac fluoroscopy and CT patients, however, a 1.3 to 1.7 fold up-regulation was still evident in some patients with a significant up-regulation at 2 hr in diagnostic CT patients.	O'Brien et al. 2018
Pediatric patients exposed to X-rays during interventional cardiology procedures	<i>in vivo</i>	0.002 – 8.004 mSv	X-rays	24 h	qPCR	No correlation between FDXR expression and dose.	Spyratou et al. 2022
Lung, neck, brain, pelvis cancer	<i>in vivo</i>	0.038–0.169 Gy	X-rays	2 h, 6–8 h, 16–18 h, 24 h	qPCR	Average fold change across all patients (and	Cruz-Garcia et al. 2022

patients undergoing radiotherapy						all doses) 24 h post exposure significantly higher compared to pre exposure.	
Patients undergoing positron emission tomography–computed tomography scan (PET-CT) and skeletal scintigraphy	<i>in vivo</i>	4.66-5.09 mSv (scintigraphy patients); 10.78-15.95 mSv (PET-CT patients)	⁹⁹ mTc-MDP (scintigraphy patients); ¹⁸ F-FDG; ¹⁸ F-PSMA; ⁶⁸ Ga-DOTATATE (PET-CT patients)	2 h	qPCR	No statistically significant change in FDXR expression.	López- Riego et al. 2023
Irradiated <i>ex vivo</i> blood from healthy donors.	<i>ex vivo</i>	0.005 Gy – 4 Gy	X-rays	2 h, 24 h	qPCR	Statistically significant upregulation of FDXR at 24 h time point after 50 mGy but not lower doses. Result from three independent experiments performed using blood from single donor.	Manning et al. 2013
CD4 ⁺ T lymphocytes	<i>ex vivo</i>	5 – 500 mGy	γ-rays	2.5 h, 5 h, 7.5 h, 10h	microarrays	Statistically significant upregulation of FDXR	Nosel et al. 2013

isolated from irradiated <i>ex vivo</i> blood from healthy donors.						after 10 mGy (5 h, 7.5 h), 25 mGy (5 h, 7.5 h, 10 h), 50 m Gy (5 h, 7.5 h), 100 mGy (all time points), 500 mGy (all time points).	
Irradiated <i>ex vivo</i> blood from healthy donors.	<i>ex vivo</i>	9.6 mSv	X-rays	1h, 6h	NGS validated by qPCR	Statistically significant upregulation of FDXR after 6h.	Kaatsch et al. 2020
Irradiated <i>ex vivo</i> blood from healthy donors.	<i>ex vivo</i>	Volume CT Dose Index (CTDIvol) 15.79–18.26 mGy; Effective dose 7 mSv	X-rays	6 h	NGS validated by qPCR	Statistically significant upregulation of FDXR.	Kaatsch et al. 2021
Irradiated <i>ex vivo</i> blood from healthy donors and prostate cancer patients.	<i>ex vivo</i>	50 mGy	X-rays	1 h	qPCR	No statistically significant change in FDXR expression.	Howe et al. 2021
Irradiated <i>ex vivo</i> blood from healthy donors.	<i>ex vivo</i>	2.6-49.7 mGy	X-rays	6 h	qPCR	Statistically significant upregulation of FDXR expression after 22.6	Schüle et al. 2023

						and 49.7 mGy.	
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