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: GTACAACGGGCTTCCTGAGA  Primer R: CTCAGGTGGGGTCAGTAGGA  Probe: CGGGCCACGTCCAGAGCCA | TaqMan assay Hs01031617\_m1 |
| Primers and probes for reference gene | TaqMan assay Hs00229263\_m1 (ITFG1) | (HPRT1)  Primer F: TCAGGCAGTATAATCCAAAGATGGT  Primer R: AGTCTGGCTTATATCCAACACTTCG  Probe:  CGCAAGCTTGCTGGTGAAAAGGACCC | TaqMan assay Hs99999901\_s1  (18S rRNA) |
| PCR conditions | 1x/95°C/10min, 40x/95°C/15s, 60°C/1min | 1x/95oC/2min,  45x/95oC/10s, 60oC/1min | 1×/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 | *ex vivo*/*in vivo* | Dose | Radiation type | Time points | Method | Main Findings | Reference |
| Cardiac fluoroscopy patients and CT patients. | *in vivo* | 9-76 mSv (Cardiac Fluoroscopy Patiens);  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 | *in vivo* | 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 patients undergoing radiotherapy | *in vivo* | 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 all doses) 24 h post exposure significantly higher compared to pre exposure. | Cruz-Garcia et al. 2022 |
| Patients undergoing  positron emission tomography–computed tomography scan (PET-CT) and skeletal scintigraphy | *in vivo* | 4.66-5.09 mSv (scintigraphy patients);  10.78-15.95 mSv (PET-CT patients) | 99mTc-MDP (scintigraphy patients);  18F-FDG;  18F-PSMA;  68Ga-DOTATATE (PET-CT patients) | 2 h | qPCR | No statistically significant change in FDXR expression. | López‑Riego et al. 2023 |
| Irradiated *ex vivo* blood from healthy donors. | *ex vivo* | 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 isolated from irradiated *ex vivo* blood from healthy donors. | *ex vivo* | 5 – 500 mGy | γ-rays | 2.5 h, 5 h, 7.5 h, 10h | microarrays | Statistically significant upregulation of FDXR 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). | Nosel et al. 2013 |
| Irradiated *ex vivo* blood from healthy donors. | *ex vivo* | 9.6 mSv | X-rays | 1h, 6h | NGS validated by qPCR | Statistically significant upregulation of FDXR after 6h. | Kaatsch et al. 2020 |
| Irradiated *ex vivo* blood from healthy donors. | *ex vivo* | 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 *ex vivo* blood from healthy donors and prostate cancer patients. | *ex vivo* | 50 mGy | X-rays | 1 h | qPCR | No statistically significant change in FDXR expression. | Howe et al. 2021 |
| Irradiated *ex vivo* blood from healthy donors. | *ex vivo* | 2.6-49.7 mGy | X-rays | 6 h | qPCR | Statistically significant upregulation of FDXR expression after 22.6 and 49.7 mGy. | Schüle et al. 2023 |