BJRS
BRAZILIAN JOURNAL OF RADIATION SCIENCES

doi.org/10.15392/2319-0612.2025.2934
Braz. J. Radiat. Sci., Rio de Janeiro
2025, 13(3) | 01-45 | e2934
Editor: Prof. Dr. Bernardo Maranhão Dantas
Submitted: 2025-05-05
Accepted: 2025-07-03

Detection of Cosmic Radiation at Low Doses: Integrative Review of Biodosimetric Methodologies with Proposition of Aerospace Evaluation Index

Abreu Junior[a,b *], P. S.; Soares[a], R. F.; Costa[a], T. S.; Frigi[a,b], F. L.; Kalczuk[a], L.; Fernandes[a], P. C.; Federico[b], C. A.

[a]Instituto Tecnológico de Aeronáutica (ITA), 12228-900, São José dos Campos, São Paulo, Brazil.
[b]Instituto de Estudos Avançados (IEAv), 12.228-001, São José dos Campos, São Paulo, Brazil

*Correspondence: abreu@ita.br

Abstract: Galactic cosmic radiation (GCR) is the major risk in long-duration spaceflight when considering biological damage. Hence, monitoring the absorbed dose is required and biodosimetry will be considered an important tool especially in scenarios where physical dosimetry does not work. This integrative review tries to find, analyze, and compare different biodosimetric techniques capable of recording low doses of ionizing radiation (≤ 0.1 Gy) under simulation of spatial environments with respect to their operational applicability. The Aerospace Biodosimetric Performance Index is introduced to integrate the main parameters of the analysis. All results came from original articles in English available in open access published between 2019 and 2024 according to a PRISMA 2020 flowchart found in Scopus and PubMed databases and only eight studies were considered eligible for inclusion after performing a primary screening using Rayyan app. The major biodosimetric approaches recognized in the investigation are cytogenetic, molecular, and metabolic. The analysis time in the methodologies was between 2 and 70 hours, sample viability from 1 day to 2 years, and sensitivity as low as 0.05 Gy for the minimum detectable dose in some methods. The Yeast Metabolic Assay showed good applicability by combining high sensitivity with quick analysis and long viability. However, an absence of studies combining space radiation with microgravity constituted a major drawback. While many methodologies show detection capabilities at very low dose levels, few of them meet both the technical and operational requirements when considered together for prolonged space missions, hence a development challenge for hybridized yet more robust methods.

Keywords: Space biodosimetry, cosmic radiation, biodosimetric index.

BJRS
BRAZILIAN JOURNAL OF RADIATION SCIENCES

doi.org/10.15392/2319-0612.2025.2934
Braz. J. Radiat. Sci., Rio de Janeiro
2025, 13(3) | 01-45 | e2934
Editor: Prof. Dr. Bernardo Maranhão Dantas
Submitted: 2025-05-05
Accepted: 2025-07-03

Detección de Radiación Cósmica a Bajas Dosis: Revisión Integradora de Metodologías Biodosimétricas con Propuesta de Índice de Evaluación Aeroespacial

Resumen: La radiación cósmica galáctica (GCR, por sus siglas en inglés) es el principal riesgo en los vuelos espaciales de larga duración cuando se considera el daño biológico. Por lo tanto, es necesario controlar la dosis absorbida y la biodosimetría se considerará una herramienta importante especialmente en escenarios donde la dosimetría física no funciona. Esta revisión integradora trata de encontrar, analizar y comparar diferentes técnicas biodosimétricas capaces de registrar bajas dosis de radiación ionizante (≤ 0,1 Gy) bajo simulación de ambientes espaciales con respecto a su aplicabilidad operacional. Se introduce el Índice de Rendimiento Biodosimétrico Aeroespacial para integrar los principales parámetros del análisis. Todos los resultados provienen de artículos originales en inglés disponibles en acceso abierto publicados entre 2019 y 2024 según un diagrama de flujo PRISMA 2020 encontrado en las bases de datos Scopus y PubMed y solo ocho estudios se consideraron elegibles para la inclusión después de realizar un cribado primario con la aplicación Rayyan. Los principales enfoques biodosimétricos reconocidos en la investigación son citogenéticos, moleculares y metabólicos. El tiempo de análisis en las metodologías fue de entre 2 y 70 horas, la viabilidad de la muestra de 1 día a 2 años, y la sensibilidad tan baja como 0.05 Gy para la dosis mínima detectable en algunos métodos. El ensayo metabólico de levadura mostró una buena aplicabilidad al combinar una alta sensibilidad con un análisis rápido y una viabilidad prolongada. Sin embargo, la ausencia de estudios que combinaran la radiación espacial con la microgravedad constituyó un inconveniente importante. Si bien muchas metodologías muestran capacidades de detección a niveles de dosis muy bajos, pocas de ellas cumplen los requisitos técnicos y operacionales cuando se consideran conjuntamente para misiones espaciales prolongadas, por lo que es un desafío para el desarrollo de métodos híbridos pero más robustos.

Palabras clave: Biodosimetría espacial, radiación cósmica, índice biodosimétrico.

1. INTRODUCTION

Galactic cosmic rays (GCRs) are one of the major space travel risks when prolonged mission exposure is considered because GCRs can induce DNA damage and consequential biological effects. Ionizing radiation can not only cause genomic instability and future cancer risk[1], [2], [3] enhancement but also cognitive dysfunction[4], hematological diseases[5], circulatory diseases, cataracts, hypothyroidism, and death[6], [7]. Thus, it can produce direct damage to DNA and secondary oxidative stress by increased production of reactive oxygen species and reactive nitrogen species[8].

In addition, the radiation severity coincides with a dose measure in terms of "linear energy transfer" or LET which indicates the average energy that a charged particle transfers to the medium it moves through, per unit distance traveled[9]. Accordingly, on Earth, low LET radiation (X-rays, beta and gamma rays) dominates and in space, high LET radiation has more predominant effects on humans and can produce ion and radical clusters [10].

Ionizing radiation effects are the reasons why dosimetry quantifies the interaction of radiation in matter, providing a physical measure relating to current or potential risk delivered to an individual. This is therefore a measure that ICRP (International Commission on Radiological Protection) and ICRU (International Commission on Radiation Units and Measurements) have developed, which indicates humans and other organisms quantitatively about their exposure to radiation [9], [11].

The relationship between dose and response with the effects of radiation particularly in human tissues has been used not only to ensure that the doses administered in medical procedures are accurate, safe, and effective [12] but also used in the development of predictive models for biological response to radiation which are fundamental in defining protection protocols calibrating equipment for radiotherapy as well as new treatments based on radiation[13].

At present, the evidence that dosimetry practice correlates with patient safety is mounting[14], [15], [16]. But in most situations of actual or suspected accidental exposure to ionizing radiation, it is not possible to carry out physical dosimetry for retrospective estimates. Alternative biomarkers have thus been proposed in such situations[17], [18], [19], [20]. So, in response to this demand for techniques that can better evaluate radiation effects, particularly on humans, "biodosimetry" or "biological dosimetry" is called upon to play a central role in the field of biological protection. The absorbed radiation dose can be evaluated by compared ways from physical dosimetry and biodosimetry. Physical dosimetry is the field concerned with measuring ionizing radiation absorbed by non-living materials[21]. This is done using Thermoluminescent Dosimeters (TLDs), ionization chambers, or semiconductor detectors[22], [23] while evaluating physical properties such as electron paramagnetic resonance[24] (EPR) and optically[25] or thermally stimulated luminescence[26] (OSL, TL). Those are possible of recording luminescence based localized traces of exposure to radiation and thus rather indirect in reflecting the actual absorbed dose by an organism, particularly under non-homogeneous irradiation conditions[27].

The term biodosimetry applies to the observation of biological changes resulting from radiation exposure and includes breaks in DNA as well as chromosomal aberrations detectable through such techniques as DCA [28] (Dicentric Chromosomal Assay), CBMN [29] (Cytokinesis Blocking Micronucleus Assay, and FISH[30] (Fluorescence In Situ Hybridization). It may also depend on the analysis of damage-response gene and protein expression using γ-H2AX[31] and GE[32] (Gene Expression) that better reflect radiation interactions with the body since they consider the direct biological effect of radiation on living tissues[27]. Biodosimetry is the biological measure of the radiation dose an individual has received through accidental exposure[17], [33], particularly when physical dosimetry is not available or there is uncertainty about the incident[34]. It is a dose-response curve based between the absorbed radiation and the biological markers used and will complement the clinical dosimetry that must be performed hours and days after exposure[35]. These may be

clinical symptoms recorded as daily blood cell counts or central nervous system function such as alopecia, vomiting and diarrhea[36] or genomic, metabolic, or protein changes in biological entities [33], [37] that provide information for categorizing doses (e.g., <1 Gy, 1-2 Gy, 2-6 Gy, >30 Gy), but also for therapeutic management as well as population screening. Biodosimetry can indicate the dose of exposure, which helps in making therapeutic decisions while also assessing long-term risks[17], [36], [38].

Since clinical symptoms and changes in imaging tests can take months to manifest after radiation exposure, making them weak markers for intervention[20], several biodosimetric methodologies have been studied and evaluated for their efficacy to estimate the dose of radiation absorbed by exposed individuals, being the quantification of dicentric chromosomes (*Dicentric Chromosome Assay* - DCA) the biodosimetric method known as the gold standard to date[28], [33]. As described by Balajee et al. (2023), these approaches may be grouped into five principal categories: prodromal signs and symptoms which reflect the visible body's immediate responses to radiation, hematological analyses such as lymphocyte depletion kineticsand neutrophil/lymphocyte ratio, cytogenetics including dicentric assays (DCA), Cytokinesis-Block Micronucleus Assay (CBMN), premature chromosomal condensation(PCC) and γ-H2AX assay, or even more genomics, transcriptomics, proteomics, metabolomics large-scale probing changes in molecular profiles and physical dosimetry like for instance electron paramagnetic resonance imaging EPR)[33].

Biodosimetric methodologies are key to the assessment of astronaut exposure and the subsequent development of mitigation strategies[7], [27].

Notable biodosimetric techniques that allow direct assessment of DNA damage are the DCA [28], the CBMN [39], and γ-H2AX [40] detection and the rest will be considered. Damage in the form of double helix breaks caused by ionizing radiation is what DNA suffers in the space environment when it is most applicable [20], [41], since high-energy ionizing radiation comprises mostly heavy ions [42], [43].

In addition to regular cytogenetic testing, omics approaches such as transcriptomics, proteomics, and metabolomics emerge as extra useful measures, showing more clarity in discovering small biological changes related to cosmic ray exposure [37], [44]. Putting these methods together can greatly increase the verification of how radiation affects the cell and tiny molecule levels [45].

But whether these methods can be applied in aerospace situations depends on many factors, such as sensitivity, specificity, response time, and whether they can work in an environment with limited resources [46].

Considerable progress has been made, but there is still a gap in identifying methodologies capable of detecting the lower levels that can be anticipated for extended deep space missions[47]. Thus, from very elementary DNA breaks to more complicated chromosomal rearrangements caused by cosmic radiation interference, there is a strong demand for various complementary biodosimetric methods. For this reason, methods that bring together different markers, such as integrated micronucleus assays and γ-H2AX evaluation, have been proposed as less fallible [33].

Another factor still to be addressed is that, although progress has been made in the field, biological research on organisms beyond low Earth orbit (LEO) missions is minimal due to the long pre-launch periods during which the biological payload has to remain for months in controlled or uncontrolled conditions, as well as extended mission durations. Limit laboratory choices to model organisms can maintain inoperable states for long periods [48]. And although previous reviews have been made on biodosimetry, an integrative analysis has not been found in the literature comparing specific methodologies for exposure to cosmic radiation at low doses (≤0.1 Gy), an imperative scenario for upcoming space missions, since significant deterministic effects, such as immunosuppression of the thymus and adenoid glands, can start as early as this dose limit [49] or even lower doses can, theoretically trigger stochastic effects like cancer and hereditary diseases [9].

For the above reasons, an integrative review shall be undertaken to assess, in a careful and contrasting manner, the biodosimetric techniques presently applicable, with particular emphasis on their practical application in aerospace environments. Thus, this integrative review will try to identify, assess, and contrast the major biodosimetric techniques used for detecting cosmic radiation with regard to detection limits, sensitivity or strength, and practical easiness concerning aerospace exploration. Strategies based on cellular and molecular biomarkers like dicentric chromosome (DCA), micronucleus (CBMN), γ-H2AX, transcriptomics, metabolomics etc., were investigated to answer: "What are the most used biodosimetric methodologies today and which of them have greater applicability in aerospace exploration?". This work also proposed the Aerospace Biodosimetric Performance Index (ABPI) as a means of evaluating the methods here considered.

2. MATERIALS AND METHODS

This review was carried out in accordance with PRISMA 2020 rules to allow transparency and standard among steps taken for data choice, analysis, and mixing. The whole process used for screening and choosing articles was done in a double-blind way with help from two other researchers (P.A. and R.F.) plus the Rayyan program[50]. Any differences were solved by agreement between these two researchers Descriptive stat analyses and graph making were done using R Studio software (2024 version) using plotly, ggplot2 ,GGally dplyr, reshape2 , and RColorBrewer packages.

2.1. Eligibility Criteria

Studies were selected based on the following criteria:

2.1.1. Inclusion Criteria

- Published between 2019 and 2024.

- Original, open access articles written in English.
- Studies that use biodosimetric methodologies to measure ionizing radiation and that directly answer the guiding question.
- Studies that explicitly mention in the title or abstract the use of biodosimetric methodologies in the context of ionizing radiation.

2.1.2. Exclusion Criteria

- Articles with more than 5 years of publication.
- Literature reviews, theoretical articles without experimental data, and studies based only on simulation or modeling.
- Studies focusing on pharmacological evaluation.
- Studies in languages other than English.
- Studies that do not explicitly mention at least one biodosimetric methodology in the title or abstract.
- Studies that only address X-rays, ultraviolet, or biosignatures.
- Research involving animal models.
- Methodologies that did not test absorbed doses equal to or less than 0.1Gy.

These criteria were established to ensure that the selected articles are directly related to the scope of the review and provide robust evidence on the biodosimetric methodologies applicable to the aerospace environment.

2.2. Sources of information and research strategy

The bibliographic search was carried out in widely recognized databases:

- SCOPUS
- PubMed

2.3. Research strategy

Boolean combinations of terms related to biodosimetry and cosmic radiation were used, such as:

(biodosimetry OR biodosimeter OR biossensor OR "biological marker" OR "biological dosimetry")

AND ("galactic cosmic radiation" OR "cosmic rays" OR "ionizing radiation")

E (method OR technique OR detection OR sensitivity)

The following filters have been applied:

- Period: 2019 to 2024.
- Language: English only.
- Access: Full-text and open access articles only.

The search was conducted on September 11, 2024, ensuring that the data collected was updated. All references were organized and managed using specific software for systematic review.

2.4. Study selection process

The screening of the articles was carried out in two phases:

1. Reading titles and abstracts
2. Reading the full text

In both phases, two independent reviewers analyzed the identified records and articles that did not meet the inclusion criteria were excluded.

Only those that satisfied the guiding question ("Which biodosimetric methodologies researched in the last five years allow measuring the effect of radiation dose in a space-like field at doses below 0.1Gy?").

2.5. Data extraction

Data extracted from each article included:

- Authorship, year of publication and title.
- Biodosimetric methodology employed.
- Type of radiation tested and range of doses used.
- Minimum detectable dose (MDD) for detection of the effect of radiation exposure.
- Average LET of the MDD.
- Dose rates used
- Test analysis time and sample viability time
- Advantages and limitations identified.

The information was organized in a structured spreadsheet to enable comparative analyses.

2.6. Assessment of risk of bias

Risk of bias was assessed using the *Joanna Briggs Institute* (JBI) [51] manual, which is suitable for integrative reviews. The criteria included:

- Clarity of the methodology used in the studies.
- Adequacy of statistical methods
- Reproducibility of the experiments.
- Potential conflicts of interest stated by the authors.

All studies were at moderate risk of bias, with the main limitations related to the heterogeneity of the doses tested, variation in the exposure methodology, and lack of standardization in the quantification of biomarkers.

2.7. Measures of effect

The results of the biodosimetric applicability were analyzed considering particularly 4 main parameters that were used in the Aerospace Biodosimetric Performance Index (ABPI), a proposition of this study and that could help in the comparisons between different biodosimetric methodologies regarding their applicability in aerospace exploration, especially long-distance. It is known that for the analysis of the diagnostic performance of different clinical methodologies there is a well-established index, which is the *Youden index* (J), widely used to evaluate the diagnostic capacity of the test, taking into account, mainly, the sensitivity and specificity for its formulation [52]. This index is given by the formula:

$$\mathbf{J = Sensitivity + Specificity - 1.}$$

This index is used to evaluate the performance of diagnostic tests, especially to find the ideal cutoff point in ROC (*Receiver Operating Characteristic)* curves. Possible values range from 0 (test with no diagnostic utility) to 1 (perfect test).

Where:

- **Sensitivity (or true positive**): proportion of correctly identified patients.
- **Specificity (or true negative):** proportion of non-patients correctly identified.

However, this index does not take into account the analysis time and the feasibility time of the sample, factors that can be crucial in determining a biodosimetric methodology applied to the aerospace environment. In addition, many biodosimetric methodologies do not make clear their respective sensitivities and specificities. Thus, the proposed index is as follows, which was used as an aid tool in this review:

$$\mathbf{ABPI} = \frac{\mathbf{1}}{\boldsymbol{Dmin}} \mathbf{x} \frac{\mathbf{V}}{\mathbf{T}} \mathbf{x} \frac{\mathbf{1}}{\mathbf{LET}+\boldsymbol{K}}$$

Where:

- $\boldsymbol{Dmin}$ (Gy) = Minimum detectable dose required to generate a measurable effect. The lower this value, the more sensitive the methodology.
- **V** (days) = Sample viability time for biodosimetric testing. Methods that allow the preservation of the sample for prolonged periods are more advantageous, which justifies their positive impact on ABPI.
- **T** (h) = Time required to obtain a result after exposure. Methods that require long periods for analysis are less efficient, so the V/T ratio favors those with high feasibility and fast response.
- **LET** (keV/μm): Linear transfer of energy from the radiation used in the experiment. Studies indicate that high-LET particles (such as heavy ions) tend to induce biological damage more easily, while low-LET radiation (such as gamma rays and X-rays) generally requires higher doses to generate detectable effects. Thus, the term 1/(LET+k) gives greater weight to methodologies with higher sensitivity, capable of detecting low LET radiation [53].
- $\boldsymbol{k}$ = Adjustment parameter (= 10) to avoid distortions in comparisons with very high LET values.

2.8. Synthesis methods

The data were qualitatively synthesized following the rules of PRISMA 2020, emphasizing the description and structured comparison of the biodosimetric methodologies included. The extracted data were tabulated for prompt comparison between the techniques considered in relation to the Minimum Detectable Dose (MDD), LET, dose rate, analysis time and sample viability.

The Aerospace Biodosimetric Performance Index (ABPI) was proposed and applied, integrating key parameters to evaluate the applicability of methodologies in the detection of

cosmic radiation within an aerospace context. Due to the heterogeneity of the study, a quantitative meta-analysis was not performed and instead a narrative synthesis was undertaken to emphasize the merits, demerits, and possible applications of each approach. The entire process has been documented for transparency and reproducibility purposes.

3. RESULTS

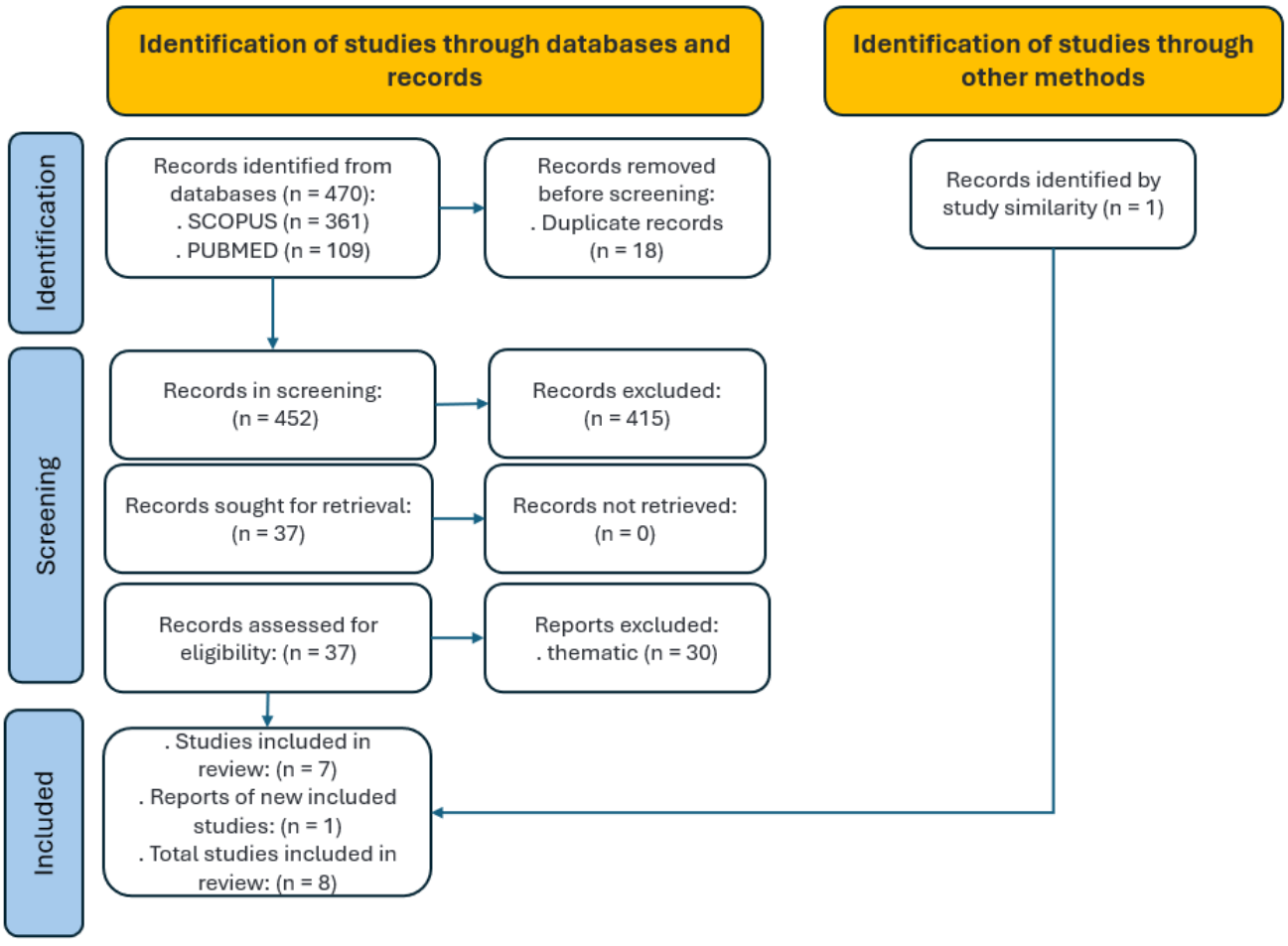
3.1. Selection of Studies

A total of 470 articles were first retrieved from searching the PubMed and Scopus databases. This left 452 articles for initial screening after removing duplicates. The selection was carried out by two independent reviewers using the Rayyan platform, and any difference was resolved by consensus among the reviewers.

Thus, 415 articles were excluded after going through their title and abstract simply because they did not fit the inclusion criteria.

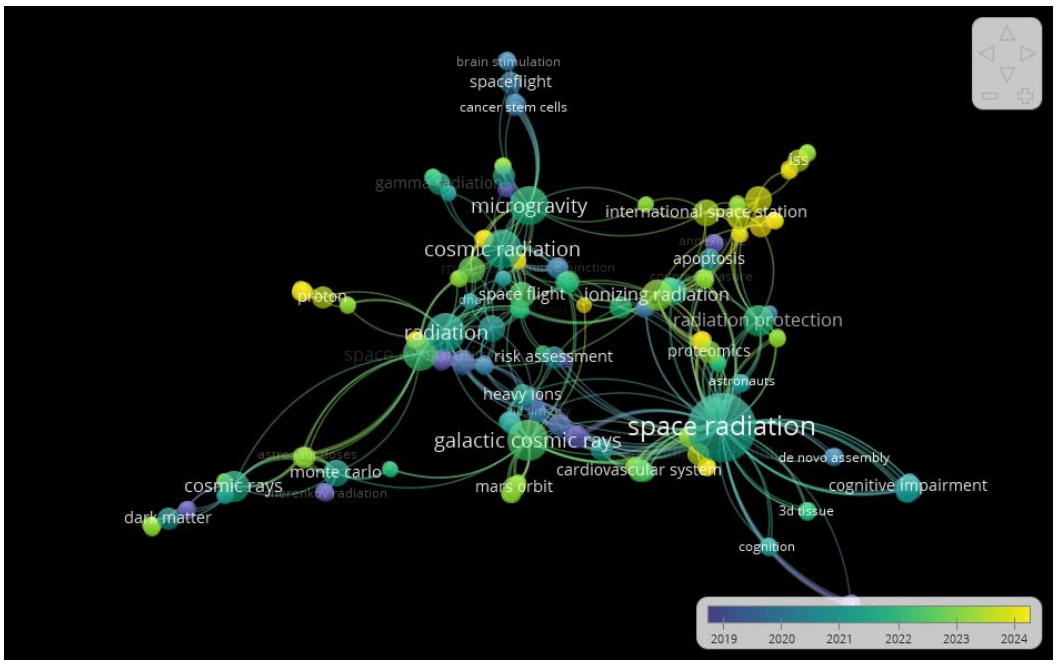
During the reading phase of the full text, 30 articles were excluded for reasons such as the biodosimetric methodologies not being applicable to cosmic radiation or because they used animal models, pharmacological models, or just computer simulations. This resulted in a situation where out of 37 articles considered eligible, once one article was added because it refers to the same project as one of the screening articles and because it completes relevant information, only 8 could be part of the integrative review.

Figure 1: PRISMA diagram. The PRISMA diagram illustrates the selection process of the studies included in this integrative review on the comparison of the minimum limits of detection of cosmic radiation in different biodosimetric methodologies. Identification: A total of 470 articles were identified in the SCOPUS (n=361) and PubMed (n=109) databases. After the removal of 18 duplicate records, 452 articles remained for screening. Screening: During the reading of titles and abstracts, 415 articles were excluded because they did not meet the inclusion criteria, resulting in 37 publications eligible for reading in full. Eligibility: After reading the questionnaire, 30 articles were excluded because they did not specifically address biodosimetric methodologies for cosmic radiation or because they did not meet the established criteria. Inclusion: A total of 8 studies were included in the review, with one of them being added later to complement information from one of the 7 studies. Details of exclusions: wrong population (n = 147), wrong result (n = 83), animals (n = 78), simulation (n = 43), review (n = 40), wrong study design (n = 15), biosignature (n = 7), wrong publication type (n = 1), wrong methodology (n = 1).



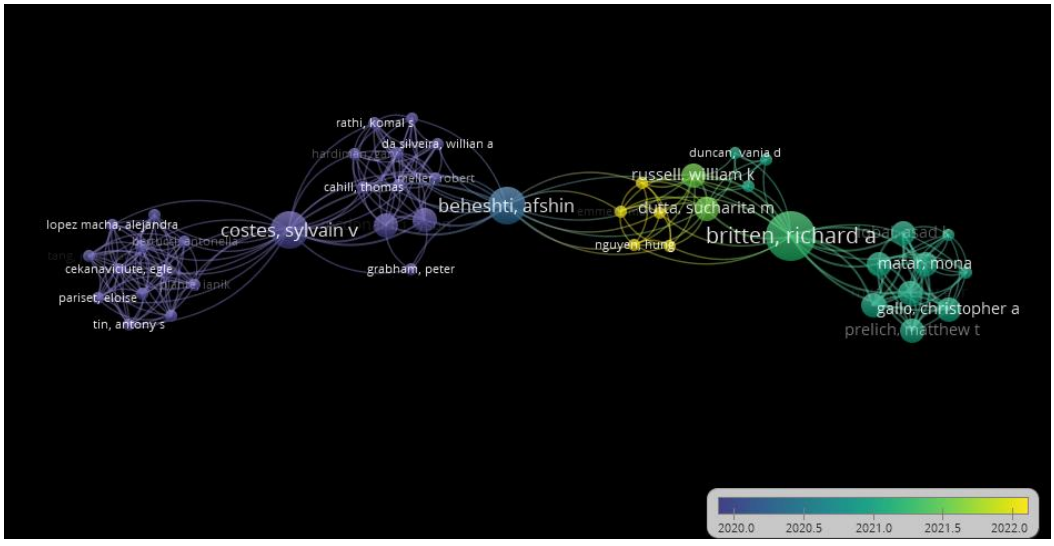
Source: The authors.

Figure 2: Authors' keywords. The map was made in the VOSviewer program and shows the frequency and relationship of the main terms that the authors use in the searches of the review. Large circles show more common keywords, such as "space radiation," "cosmic radiation," and "galactic cosmic ray." The color shows the date of the publications that go from 2019 to 2024, showing the change in research on radiation in space, low gravity, biodosimetry and live effects on astronauts.



Source: The authors.

Figure 3: Diagram autodescription excessive visualization (by the authors). The co-authorship map, also generated in VOSviewer, illustrates the connection between the most relevant researchers in the research carried out.



Source: The authors.

3.2. Study characteristics

This article maps out eight experimental studies carried out between 2019 and 2024, all geared towards biodosimetric methodologies capable of identifying ionizing radiation doses less than or equal to 0.1 Gy in environments that were rough simulations of the aerospace environment. The methodologies compared in these works were biodosimetric approaches from the classical ones based on chromosomal damage to newer molecular, omics, and metabolomics techniques which may cater to the objective of tracking cosmic radiation concerning their applicability and limitations. Mostly, they are laboratory tests performed with human cells and germ models that were hit by strong LET particle beams that included protons as well as heavy ions like iron, titanium, and boron neutrons and occasionally gamma rays. These tests were carried out in specialized laboratories equipped with radiation sources capable of mimicking aspects of cosmic radiation.

The biodosimetric methodologies described can be grouped into four major categories: (i) Cytogenetic, including the chromosomal aberration assay (dicentrics, rings, translocations) and the micronucleus assay(CBMN); (ii) Molecular and Proteomics, such as the quantification of phosphoproteins(γ-H2AX,pATF2,pSMC1)and foci of DNA damage (53BP1); (iii) Omics and Epigenetics, using epigenetic clocks based on DNA methylation (DNAmAge,epiTOC2,MiAge); and (iv) Metabolic-included AlamarBlue cell viability tests for monitoring alterations in cellular metabolism. The minimum detectable doses(MDD)dosed were from 0.05 Gy to 0.1 Gy while the dose rates ranged from 0.0129 Gy/min to 1.2 Gy/min.The comparison between the methodologies was based on Aerospace Biodosimetric Performance Index(ABPI), MDD radiation used average LET analysis time sample feasibility specific advantages limitations each technique some general characteristics studies presented below.

Table 1 : General characteristics of the studies.

AUTHOR/YEAR	TITLE	BIODOSIMETRIC METHODOLOGY	RISK OF BIAS (JBI)	DATA ACCESSIBILITY	DOES IT ANSWER THE GUIDING QUESTION?
Liddell *et al.*, 2023 [48]	BioSentinel: Validating the Sensitivity of Yeast Biosensors to Relevant Deep Space Radiation	Yeast Metabolic Assay (AlamarBlue dye)	Moderate	Open access (Astrobiology journal)	Yes
López Riego *et al.*, 2024 [54]	Chromosomal damage, gene expression and alternative transcription in human lymphocytes exposed to ionizing cells...	Chromosomal aberrations and gene expression	Moderate	Open access (Scientific Reports)	Yes
Sridharan *et al.*, 2020 [55]	Comparison of Signaling Profiles in the Low Dose Range After Low and High LET Radiation	Phosphoprotein flux cytometry (γH2AX, pATF2, pSMC1)	Moderate	Open access (Elsevier)	Yes

Engelbrecht *et al.*, 2021 [56]	DNA Damage Response of Hematopoietic and Progenitor Stem Cells to High LET Neutron Irradiation	Cytokinesis Blockade Micronucleus Assay (CBMN)	Moderate	Open access (Scientific Reports)	Yes
Nwanaji-Enwerem *et al.*, 2022 [57]	In vitro relationships of galactic cosmic radiation and epigenetic clocks in human bronchial epithelial cells	DNA methylation-based epigenetic clocks (DNAmAge, epiTOC2, MiAge)	Moderate	Open access (environmental and molecular mutagenesis)	Yes
Kowalska *et al.*, 2019 [58]	Production and distribution of chromosomal aberrations in human lymphocytes by particle bundles with different LET	Analysis of chromosomal aberrations (dicentrics, rings, translocations)	Moderate	Open access, radiation, and environmental biophysics	Yes
Radstake *et al.*, 2024 [59]	Radiation-induced DNA double-strand breaks in cortisol-exposed fibroblasts as quantified with the novel foci-integrated damage complexity score (FIDCS)	Focus-integrated damage complexity score (FIDCS), γ-H2AX/53BP1 colocation	Moderate	Open access scientific reports	Yes

3.3. Individual study results

The eight experiments in this review used different techniques for the biodosimetric evaluation of ionizing radiation in detecting doses of 0.1Gy or less, which also was tested against dose levels simulating cosmic radiation. The samples, human cells and microorganisms, were exposed in vitro to various high LET radiations: heavy ions (Fe, Ti, Boron), protons, and neutons as well as gamma radiation.

The longevity of samples of Saccharomyces cerevisiae observed in Liddell et al., 2023 [48] came from a prior study done by Santa Maria S et al., 2023[7] because it refers to the same Biosentinel project and proved that these samples were viable for 2 years.

Biodosimetric approaches in this context involve cytogenetic, molecular, proteomic and metabolic techniques. Their sensitivity, time to analysis, and limits of detection vary so that a pragmatic evaluation of its applicability is made in the aerospace context. An intercomparison of these methodologies is shown in the next table 2 below with the main parameters analyzed such as Minimum Detectable Dose (MDD), mean LET associated with MDD, and radiation doses and dose rates used.

Table 2: Other parameters analyzed, including Minimum Detectable Dose (MDD), mean LET associated with MDD, radiation doses, and dose rates used in the included studies.

AUTHOR/YEAR	BIODOSIMETRIC METHODOLOGY	TYPE OF RADIATION	LET (keV/μm)	RADIATION DOSES USED (Gy)	DOSE RATES	MINIMUM DETECTABLE DOSE (Gy)
Liddell *et al.*, 2023	Yeast Metabolic Assay (AlamarBlue dye)	Protons and heavy ions	0.2 (protons), 100 (heavy ions), 46.2 (medium mixed beam)	0, 0,01, 0.05, 0,1, 0,35, 0,5 and 1	NA	0.05 (Mixed Beam GCR)
López Riego *et al.*, 2024	Chromosomal aberrations and gene expression	Mixed beam (alpha and photons)	X-Ray: 0.3, Alpha: 90.9, Mixed beam (46.2)	0, 0,5, 1,0 and 2,0	0.068 (X-rays), 0.223 (Alpha), mixed radiation (half the doses also per minute)	0,12 (Mixed beam)
Sridharan *et al.*, 2020	Phosphoprotein flux cytometry (γH2AX, pATF2, pSMC1)	Feixe mixed (Si, Fe, and Ti)	Si: 69, Fe: 239, Ti: 171. LET average: 159.67	0, 0.05, 0,1 and 0,5	0.1 Gy/min for low doses (0.05 and 0.1 Gy)1 Gy/min for high doses (0.5 Gy)	0.05

Engelbrecht *et al.*, 2021	Cytokinesis Blockade Micronucleus Assay (CBMN)	Gamma and neutrons	0.3 (range), 75.0 (neutron)	0, 0.05, 0,5 and 1,0	Gamma radiation (60Co γ-rays): 0.468 Gy/minNeutron radiation (p(66)/Be(40)): 0.400 Gy/min	0.05 (range), 0.05 (neutron)
Nwanaji-Enwerem *et al.*, 2022	DNA methylation-based epigenetic clocks (DNAmAge, epiTOC2, MiAge)	Iron	Fe-56:165	0, 0,1, 0,3 and 1,0	0,1 Gy/min to 0,1 Gy0,3 Gy/min to 0,3 Gy1 Gy/min to 1,0 Gy	0,1 (Fe-56)
Kowalska *et al.*, 2019	Analysis of chromosomal aberrations (dicentrics, rings, translocations)	Boro	76 (Boro)	0.05, 0,1, 0,2, 0,5, 1,0 and 2,0	1.2 Gy/min for all doses tested.	0.05 (boro)
Radstake *et al.*, 2024	Focus-integrated damage complexity score (FIDCS), γ-H2AX/53BP1 colocation	Gamma and iron	0.3 (gamma), 155 (iron)	0, 0,1, 0,5 and 1,0	Gamma radiation (137Cs γ-rays): 0.008 Gy/s (~0.48 Gy/min)Iron-56 radiation (1 GeV/n): For 1.0 Gy, the fluence was 4 × 10^6 ions/cm² (~0.5 Gy/min)	0.1 (range), 0.1 (iron)

*NA: Not available.

3.4. Comparison of Methodologies Based on the ABPI Index

In order to integrate key parameters in the operational evaluation of the biodosimetric methods analyzed, the Aerospace Biodosimetry Performance Index (ABPI) was proposed, which takes into account the minimum detectable dose (MDD), the feasibility of the sample, the analysis time and the average LET of the MDD, on a logarithmic scale.

Thus, the results revealed different ABPI values, with emphasis on the metabolic assay with yeasts (Liddell et al., 2023), which obtained the highest value (25.98), due to the combination of high sensitivity (0.05 Gy), moderate analysis time (10 h) and, above all, prolonged viability time of the tested samples (730 days).

On the other hand, classic methodologies such as CBMN and DCA had ABPIs below 0.01. Table 3 below summarizes the ABPI values obtained for each included study.

Table 3 : The following table presents a better detail between the data used for the comparison between the biodosimetric methodologies in relation to ABPI.

AUTHOR/YEAR	METHODOLOGY	TYPE OF RADIATION	LET'S Medium (keV/µm)	MINIMUM DETECTABLE DOSE (Gy)	ANALYSIS TIME (h)	SAMPLE VIABILITY TIME (DAYS)	ABPI
Liddell *et al.*, 2023	Metabolomics	Protons and heavy ions	46.2	0.05	10	730	**25.98**
Radstake *et al.*, 2024	Foci/DNA complexity	Gamma and iron	155	0.1	0.5	2	**0.1212**
Sridharan *et al.*, 2020	Phosphoproteins	Feixe misto (Si, Fe, Ti)	159.67	0.05	2	1	**0.0589**
Engelbrecht *et al.*, 2021	CBMN	Gamma and neutrons	75	0.05	70	2.9	**0.0098**
Kowalska *et al.*, 2019	Cytogenetics	Boro	76	0.05	48	2	**0.0097**
López Riego *et al.*, 2024	Cytogenetic/Genetic	Mixed beam (alpha and γ)	46.2	0.12	24	1	**0.0062**
Nwanaji-Enwerem *et al.*, 2022	Epigenetics	Iron	165	0.1	48	2	**0.00238**

4. DISCUSSIONS

This review incorporates the Aerospace Biodosimetric Performance Index (ABPI) proposed and preliminarily tested as a quantitative tool for integrating different parameters related to the use of biodosimetric methods in the context of space missions. ABPI has not yet undergone formal validation and is considered an auxiliary tool in the comparison carried out in this review. It allowed the integration of quantitative (sample feasibility) and qualitative (advantages and limitations) data. Therefore, here it will serve as an unvalidated aid in the comparison of analyses that link quantitative measures with qualitative ones. An important consideration is that, when ABPI can provide a synthesis of objective criteria for comparing methods in its initial phase of development, it should be used with qualitative evaluations considering the technical and operational specificities of each methodology.

4.1. Sensitivity and Detection Limits of Methodologies

The capacity to sense low levels of radiation is a vital requirement for any biodosimetric method when discussing the space environment. From the techniques reviewed, only a few were able to distinguish themselves due to their higher sensitivity towards identifying low doses of radiation linked with DNA harm and these include the γ-H2AX, pATF2 and pSMC1 assay which could register up to 0.05 Gy and also showed a fairly quick analysis time, under 2 hours [55]. These methods rely on the finding and investigation of the phosphorylation of particular proteins with the DNA damage response, like γH2AX pATF2 and pSMC1, using flow cytometry to check the strength of phosphoprotein signaling in cells hit by radiation. These markers showed not only speed but also the ability to find exact damage, symbolizing an important instrument for cases when time to respond matters [55].

Furthermore, the CBMN [56] that Engelbrecht et al. (2021) reviewed also showed high sensitivity ~0.05 Gy albeit with long analysis time ~70 hours CBMN depends on detection of whole or broken chromosomes not incorporated into daughter nucleus at time cell division. It

acts genotoxic damage chromosomal instability Robustness international recognition validated method [60] guarantee value retrospective analyses but still limit emergency application field long analysis time. The omics and epigenetic approaches which involves epigenetic clocks DNAmAge epiTOC2 MiAge were results from the study of Nwanaji-Enwerem et al. These results showed potential in the detection of cosmic radiation but at levels that are cumulative and somewhat blunt. The epigenetic clocks DNAmAge epiTOC2 and MiAge are DNA methylation-based biomarkers and they are predictors of biological cell age and correlate well with aging processes as well as cancer risk. Therefore, in this study, a relationship was established between these clocks and GCR how different doses of ionizing radiation specifically act on these biomarkers. Only epiTOC2 registered notable acceleration also in high Fe-56 LET radiation and cumulative data showed a dose-proportional increase. This means that higher doses give higher biological estimates for cell division.

This may raise the risk of cell damage and subsequently cancer due to radiation. The ability of 0.1 Gy doses of Fe-56 to induce sensitivity does not hold in practical terms because then the analysis time stretches to about 48 hours and standardization for different radiations is not feasible [57].

The metabolomic assay with Saccharomyces cerevisiae strains as proposed by Liddell et al. (2023) [48] registered metabolic change at 0.05 Gy, within an analysis time of 10 hours, hence combining sensitivity with operational viability as will be elaborated later. A biosensor based on metabolic reduction of the alamarBlue dye was shown herewith, through 47 strains of Saccharomyces cerevisiae, to reflect the dose of absorbed ionizing radiation,, showing a dose-dependent response that was more pronounced for rad51Δ mutant strain which is known to be more sensitive to DNA damage.

4.2. LET and Type of Radiation: Impact on Methodological Choice

Cosmic radiation is a high-LET particle composed of Fe, Si, Ti, and Boron among others. Low-radiation LET is gamma photons. Biodosimetric methodologies should thus

apply to the spectrum of LET [61]. From studies in this review, methods based on quantification of phosphoproteins like γ-H2AX and micronucleus assay (CBMN) generally showed good sensitivity for radiation with LET from 0.3 up to 239 keV/μm. [55], [56]

However sensitivity results based on the formation of dicentric chromosomes and chromosomal aberrations were limited as per Kowalska et al., 2019 [58] and were sensitive to 0.05 Gy Boron which is 76 keV/μm. The limitations in resolving mixed and complex radiations, typical of a space mission, arise because these techniques cannot resolve damage patterns caused by mixed and highly ionizing radiations. This is due to the fact that spatial distribution and energy deposition statistics – as reflected by the difference between Poisson and Neyman A models – produce considerable heterogeneity of cellular response for accurate attribution of incident radiation quality and dose. Analysis of chromosomal aberrations and dicentric chromosomes, very sensitive though well-standarded against cosmic radiation [33], [62], requires relatively long time for analysis (24 to 48 hours) along with some methodological complexity which may pose problems for application in space.

The methodology was acknowledged for the novel ability of the FOCI-Integrated Damage Complexity Score (FIDCS) [59] to assess structural complexity as a parameter in resistance against high LET radiation, specifically Fe-56, measured at 155 keV/μm. Essentially, this is an assessment that just multiplies area and fluorescence intensity for each DNA damage focus and integrates these values by cell nucleus in order to quantify more accurately damage induced by ionizing radiation than simply by counting foci. It has shown sensitivity to the best of its ability only for doses greater than 0.1 Gy so that may limit its application to very low doses but just by showing the ability to differentiate damage complexity it stands well as a method in mixed radiation and high LET environments.

4.3. Operational Aspects: Analysis Time, Feasibility and Spatial Applicability

For practical application in space missions, biodosimetric methodologies should ideally be fast, stable, and compatible with the space environment [34], [63]. In this sense the metabolomic methodology recommended by Liddell *et al.* [48] presented the best operational profile integrating high sensitivity (0.05 Gy) reduced analysis time (10h) and prolonged viability of the samples (2 years in the dry state) making its use in long-duration missions feasible.

Another thing, old ways like CBMN [56] and DCA [58], though strong, might need tricky lab setups and long wait times, making it hard to use them right on site, except with big changes using optimization or automation tech. Molecular strategies like phosphoprotein-quantification [55] have demonstrated rapid analysis times (maximum of 2 hours), but with a Constraint of the low viability time for the samples (24-48h), which thus limits their use for analysis to be done right after exposure.

4.4. Comparison by ABPI: Impact on the Choice of the Best Methodology

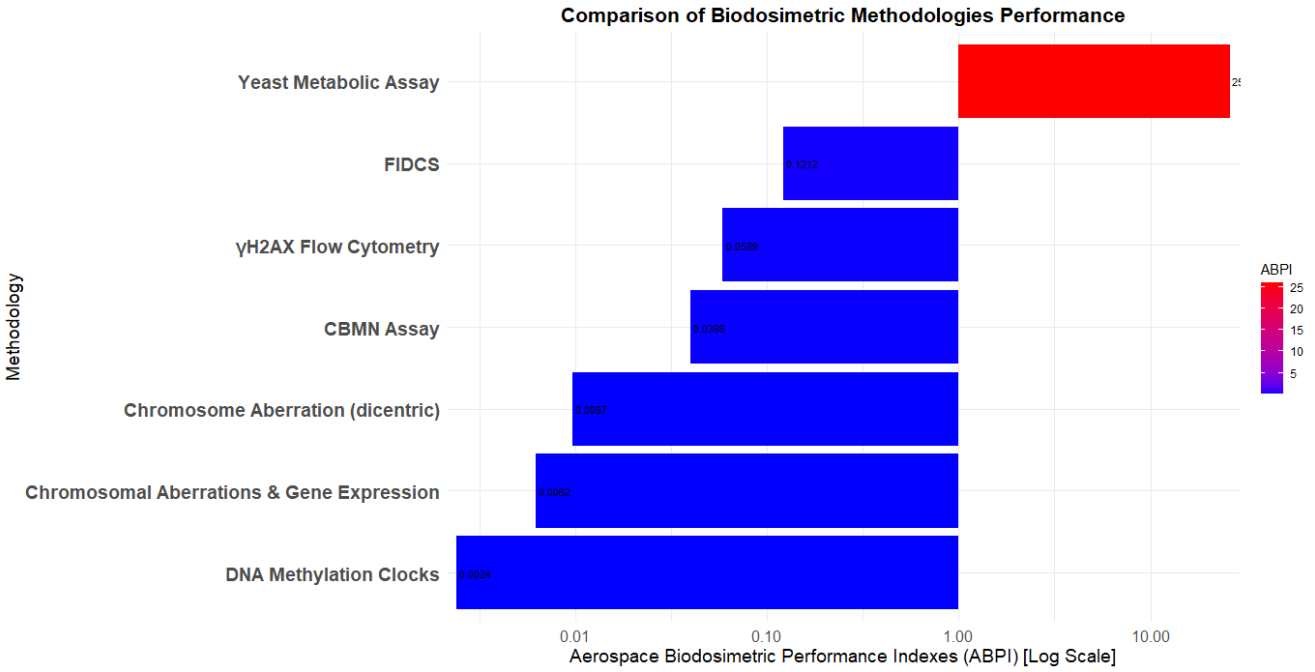
Though the application of ABPI as a preliminary comparative tool has exposed the Yeast Metabolic Assay [48] as one with excellent operational applicability, it should be noted that other input and equipment costs were not considered as variables in obtaining the proposed index and hence it is recommended to use such other factors alongside ABPI for a broader view on how method applies.

Other forms gave average ABPIs showing good sensitivity but also practical limits like need for better microscopy or cytometrym like FIDCS phosphoprotein quantification [59].

CBMN and DCA, although potent, recorded the lowest ABPIs. This is fundamentally a reflection of the drawback of long analysis time which could be offset by techniques to optimize this time so that these techniques can become more applicable in aerospace exploration, since they showed fairly good sensitivity (MDD = 0.05Gy).

Therefore, using the measure recommended in the details from the included studies, where a higher ABPI means greater possibility for the approach to be applicable in the aerospace situation, we get figure 4 below:

Figure 4: Comparison of the performance of biodosimetric methodologies.



Source: The authors.

The above figure shows the comparison of Aerospace Biodosimetric Performance Indices (ABPI) between different biodosimetric methodologies analyzed in this review, using a logarithmic scale that will better visualize the differences between the values obtained. It gives a first good look at how well each method works when tested in important parts of the space environment—low dose sensitivity, how it works with other things, turnaround time, and sample viability. Large differences seen between yeast metabolic assay and resting methods reflect best possible combination of high sensitivity at doses as low as 0.05 Gy, short analysis time (10 hours), and quite extended sample viability time (730 days).

Thus, the other approaches recorded ABPIs beneath 1, signifying substantial constraints in regard to the operational standards of the aerospace setting. The FIDCS, proposed by Radstake et al. (2024), attains the second position with an ABPI of 0.1212 because

it can evaluate damage complexity for high LET radiation with high specificity but is limited in terms of sample viability and analysis time as stated in minutes and hours respectively.

Methods derived from molecular biomarkers of DNA harm- like γ-H2AX, pATF2, and pSMC1 using flow cytometry- sit at medium level with ABPI of 0.0589. Though quick (2 hours) and sharp (sees 0.05 Gy) these ways their practical use gets cut by how soon the samples last (1 day) which limits their role in long space trips or times when lab setup is not right away.

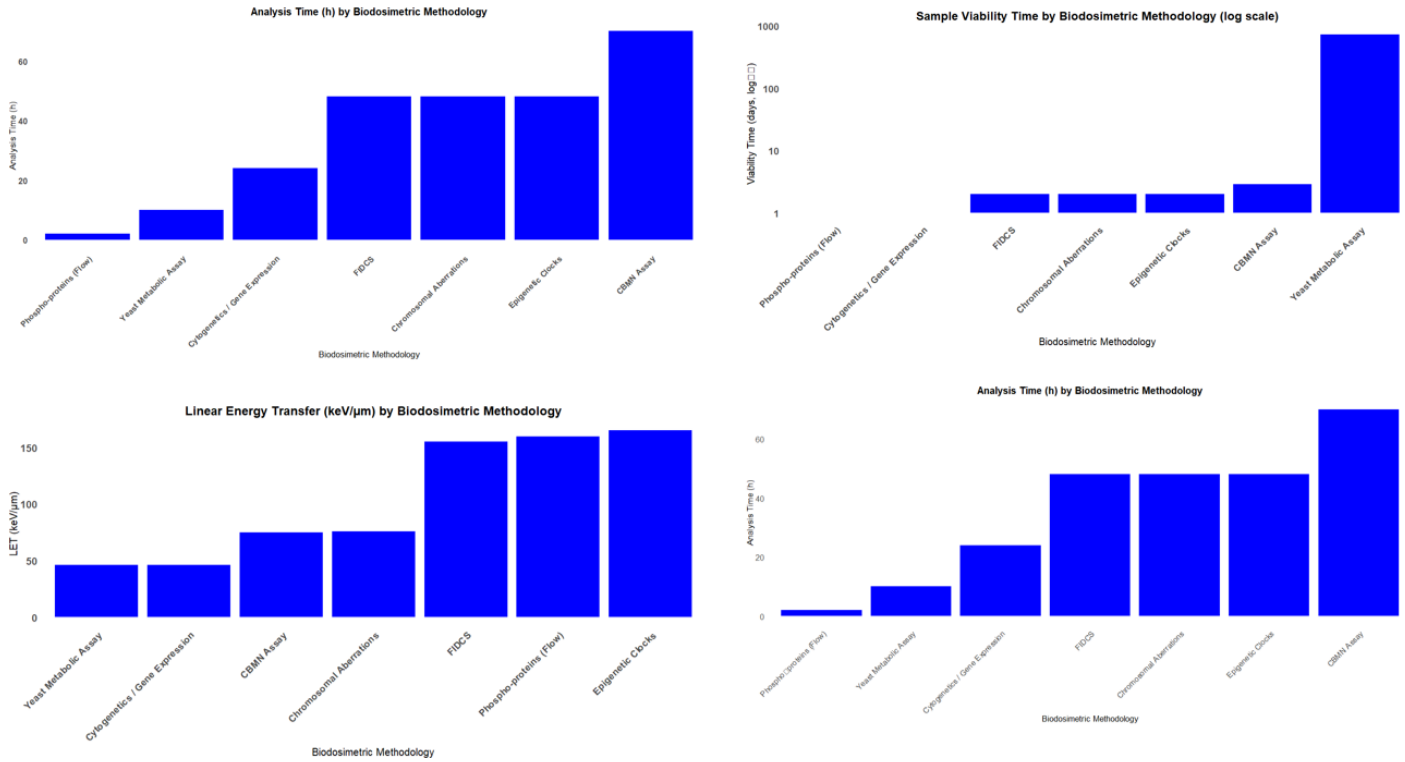
The CBMN methodology reinforces this trend with an ABPI of 0.0098. This methodology, though sensitive to even lower doses (0.05 Gy) and with a little longer sample viability time than most methodologies, had its ABPI relatively affected by the prolonged analysis time which is nearly 70 hours.

Traditional cytogenetic methods like analysis of chromosomal aberrations had one of the lowest ABIs (0.0097) because it took a long time to perform the analysis (around 48 hours) and also needed much labor, though andin high sensitivity.

The joint methods of chromosomal damage and gene expression by López Riego et al. and the time-based ages from Nwanaji-Enwerem et al. come at the lowest ABPIs (0.0062 and 0.00238, respectively) which shows major limits both in how much can be detected at low levels and in how fast they work and practical use, even though they might have a big part in checking the lasting biological effects of cosmic radiation.

Then, this finding strengthens the need to focus on creating new biodosimetric methods that balance sensitivity with operational practicality, mainly for situations of extended space missions.

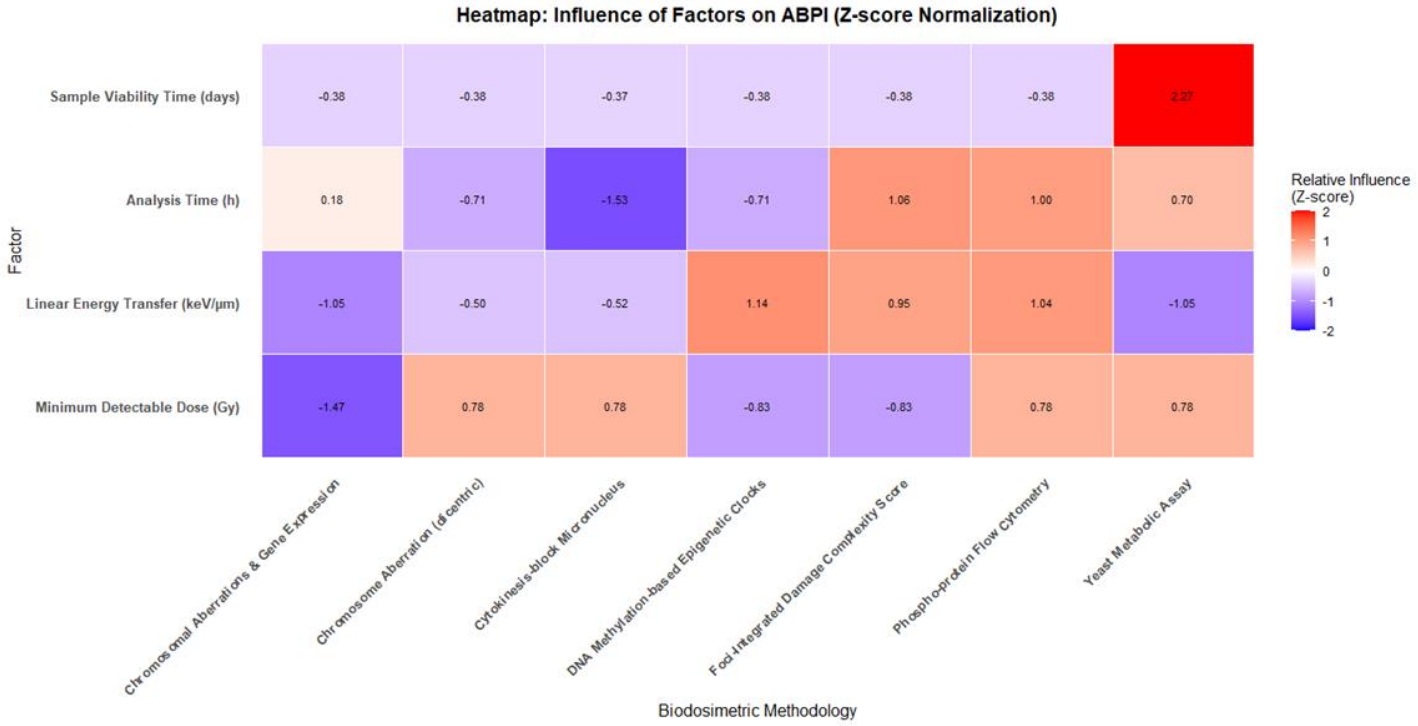
Figure 5: Summary of the results of the main parameters evaluated in the different study methodologies (MDD, mean LET, sample viability time, and analysis time).



Fonte: Os autores.

For a better evaluation and visualization of the influences of each of the 4 factors (MDD, mean MDD LET, analysis time, and sample viability) on the ABPI of each biodosimetric methodology evaluated, a heat map was generated from the normalization of the data by the Z-score, which is represented in figure 6 below.

Figure 6 : Heat map: Influence of factors on ABPI (normalization of the Z score).



Fonte: Os autores.

The Z-score normalization heat map enabled a single-file view of the relative impacts of the major constituents of the Aerospace Biodosimetric Performance Index (ABPI) across all methods analyzed. Data depicted in the chart with results from this review showed high degrees of correspondence, thereby reinforcing the value of ABPI as the primary benchmark tool in setting up this review.

The Yeast Metabolic Assay was seen in the graph as the technique with maximal positive effect on sample viability, Z-score = 2.47, reinforcing its major operational advantage already discussed in the review and that allows conservation of the sample for up to two years without loss of sensitivity, making it highly applicable for long-duration space missions. Also seen is that analysis time has little positive impact on ABPI which implies that, although it is a limiting factor for other methodologies, in this particular case of Yeast Metabolic Assay, moderate analysis time of 10 hours does not significantly compromise its performance.

The Integrated Focal and Complexity Damage Score showed a strong positive relationship with Lethal Effect Transfer, Z-score = 1.2, thus reinforcing its specificity for inducing complex damage by high LET radiations, such as Fe-56. This trend basically confirms what has been reviewed above about its potential in assessing the complexity of genetic damage in mixed cosmic radiation scenarios.

The study tried to make longer the analysis time and sample viability, proving that these variables still mostly limit their use in spatial scenarios. The sensitivity of phosphoprotein flow cytometry and Foci 53BP1+-based methods for high LETs, low analysis time, and response to low absorbed trough doses was exemplary. Sample viability time has a negative influence (Z-score close to -0.36) which confirms that the short analysis window for these methods (24 to 48h) may limit their use in extended space missions where sample preservation critically becomes important.

Cytogenetic techniques (CBMN and DCA) have, on the contrary, persistently demonstrated a strong negative effect of analysis time on ABPI. This basically reinforces its operational limitation as long durations are required for the trials to be completed (48 to 70 hours). However, the positive effect of the Minimum Detectable Dose shows that these techniques are highly sensitive for doses up to 0.05 Gy and thus they are robust methodologies but not very feasible for rapid response in operational situations.

The epigenetic clocks, in turn, reflected a good effect of the LET and the analysis time, and a fair bad effect of the Minimum Detectable Dose. This means that these ways are smart to the bio changes made by ionizing radiation but their greater ability is mainly for high LET radiation resulting in lower performance for damage finding in low LET radiation. This trait might cut down its use in cases where contact is mostly with low-LET particles—cases that often need higher doses to show obvious effects. Also, the fairly bad impact of analysis time means that these methods still have big limits because of the long times needed for data handling and decoding.

4.5. Summary of results

The great diversity between the studies taken - with respect to the type and source of radiation, LET values, doses used, times of analysis and kinds of cells employed - is an very crucial point that should not be overlooked. This very fact also hinders direct comparison between the various studies and penalizes efforts to consolidate a standardized biodosimetric methodology in the space environment.

Another major concern is that up to now, no study has integrated exposure to simulated space radiation with microgravity. Though the methodologies have been evaluated under conditions that roughly mimic some aspects of cosmic radiation (that is, heavy ion beams and high-LET particles), none of these studies has ever looked at the combined effect of radiation and microgravity. This would be the real thing in terms of a space mission, besides possibly other factors like vibration, temperature, and pressure changes that can contaminate the biodosimeter readings, particularly during launch into space.

This disunity limits complete awareness regarding the possible biological impacts on astronauts and heightens an already urgent requirement for subsequent studies that underscore these two stressors in a unified manner. Moreover, some methodologies, though hopeful, have not been validated under practical circumstances comparable to those of a space mission. Techniques like phosphoprotein assay [55] and FIDCS [59], though they possess great sensitivity and specificity, need high-resolution cytometry and microscopy systems and their its miniaturization and adaptation for use during a flight still pose considerable technical challenges.

As promising as they are, omics and epigenetic methods have yet to undergo validation in the different contexts of radiation exposure [57] and cannot be applied directly to make them reliable biodosimeters for use in space missions.

Future research should aim at developing integrated, synthesized approaches which combine signals from chromosomal damage, molecular damage, and metabolic damage

towards the creation of simplified systems that are easily transportable, with immediate self-analytical capabilities. This will serve to surpass the present limitations and facilitate better monitoring of cosmic ray exposure under microgravity conditions. The main merits and demerits of the methodologies outlined in this review are summarized in Table 4.

Table 4 - Summary of some of the main advantages and limitations of the methods.

AUTHOR/ANO	TITLE	BIODOSIMETRIC METHODOLOGY	ADVANTAGES	LIMITATIONS
Liddell et al., 2023	BioSentinel: Validating the Sensitivity of Yeast Biosensors to Relevant Deep Space Radiation	Yeast Metabolic Assay (AlamarBlue color) (metabolomic)	High sensitivity for low doses; microgravity-compatible and storable in a dry state; Extended feasibility (up to 2 years), ideal for long space missions.	It does not distinguish specific mechanisms; reduced response in dehydrated cells.
López Riego et al., 2024	Chromosomal Damage, Gene Expression, and Alternative Transcription in Human Lymphocytes Exposed to Mixed Ionizing Radiation Found in Space	Chromosomal aberrations and gene expression (FDXR, CDKN1A, MDM2)	Detects synergy between types of space radiation; response to low doses.	High variability between individuals; difficulties in differentiating the effects of radiation and microgravity; Lower accuracy in differentiating damage types.
Sridharan et al., 2020	Comparison of signaling profiles in the low range dose após radiação LET baixa e alta	Phosphoprotein flux cytometry (γH2AX, pATF2, pSMC1)	High sensitivity for high LET radiation; detailed analysis of cellular response; fast analysis time.	Short sample viability window (24 to 48h), making it difficult to apply in long space missions.

Engelbrecht et al., 2021	DNA Damage Response of Hematopoietic and Progenitor Stem Cells to High LET Neutron Irradiation	Cytokinesis Blockade Micronucleus Assay (CBMN)	Validated method for spatial biomonitoring; differentiates photons and neutrons.	Long incubation time (48 to 70h), reducing its applicability for quick responses; it does not differentiate specific types of DNA damage.
Nwanaji-Enwerem et al., 2022	In vitro relationships of galactic cosmic radiation and epigenetic clocks in human bronchial epithelial cells	DNA methylation-based epigenetic clocks (DNAmAge, epiTOC2, MiAge)	Detects long-term cumulative radiation impacts; Useful for assessing accelerated aging in astronauts.	Applicable only to specific radiation (Fe-56); sensitive to high LET values, which may limit the detection of damage caused by low LET radiation.
Kowalska et al., 2019	Production and distribution of chromosomal aberrations in human lymphocytes by particle bundles with different LET	Analysis of chromosomal aberrations (dicentrics, rings, translocations)	Sensitive to low doses; robust for RBE estimates in space.	Time-consuming process; difficulty in differentiating types of damage in exposures to mixed and complex radiation; confidence in advanced statistical analysis.
Radstake et al., 2024	Radiation-induced DNA double-strand breaks in cortisol-exposed fibroblasts as quantified with the novel foci-integrated damage complexity score (FIDCS)	Focus-integrated damage complexity score (FIDCS), γ-H2AX/53BP1 colocation	Differentiates the complexity of DNA damage; good correlation with theoretical models of space radiation.	Underestimation of damage in high LET radiation, which may compromise its application for mixed exposures in space; variable analysis time (30 min to 48 h), impacting the reproducibility of the results; requires calibration for different radiations.

5. CONCLUSIONS

This review shows that as much as several biodosimetric methods are capable of detecting doses of 0.05 Gy, only a few fulfill the technical and operational requirements needed for extended space missions.

The Aerospace Biodosimetric Performance Index (ABPI) proved useful to compare, in an integrated way, sensitivity, LET, analysis time and sample viability though it has not yet been formally validated and it highlighted the Yeast Metabolic Assay for bringing high sensitivity together with fast response and long viability while conventional methods such as CBMN and DCA have shown good robustness they were found to have operational limitations.

A key constraint in the advancement of biodosimetry for space situations, which at the end prevents a complete grasp of the possible biological impacts on astronauts, is the absence of experiments that merge high LET radiation with microgravity or other variables – vibration, temperature, and pressure – that usually exist in a space setting. So upcoming research considering these elements in an integrated manner is required.

ACKNOWLEDGMENT

My special thanks to my advisors Dr. Cláudio Federico, from the Institute for Advanced Studies (IEAv), Dr. Liana Kalczuk, and Dr. Priscila Fernandes, from the Technological Institute of Aeronautics (ITA), Brazil, who provided insights and knowledge that greatly helped the research.

REGISTRATION AND PROTOCOL

The review protocol was registered on the Open Science Framework (OSF) platform, DOI 10.17605/OSF. IO/FHN7M, ensuring the transparency of the review process and increasing the credibility of the results. During the registration, the process of selection and screening of the studies was described in detail, as well as the methodology to be used for the comparative analysis of the detection limits of the different biodosimetric techniques.

FUNDING

This work was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES) – Brazil – Funding Code 001, by the National Council for Scientific and Technological Development (CNPq) and by the Brazilian Agricultural Research Corporation (EMBRAPA).

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

AVAILABILITY OF DATA, CODES, AND OTHER MATERIALS

All data extracted from the studies included in this review are presented in the tables of the article. The codes used in the R software for statistical analysis are available upon request to the corresponding author. No new databases or additional software were generated in the development of this review.

REFERENCES

[1] A. Bushmanov, N. Vorobyeva, D. Molodtsova, and A. N. Osipov, 'Utilization of DNA double-strand breaks for biodosimetry of ionizing radiation exposure', **Environmental Advances**, vol. 8, Jul. 2022, doi: 10.1016/j.envadv.2022.100207.

[2] M. Durante and L. Manti, 'Human response to high-background radiation environments on Earth and in space', **Advances in Space Research**, vol. 42, no. 6, pp. 999–1007, 2008, doi: 10.1016/j.asr.2007.02.014.

[3] M. Moreno-Villanueva, M. Wong, T. Lu, Y. Zhang, and H. Wu, 'Interplay of space radiation and microgravity in DNA damage and DNA damage response', Dec. 01, 2017, **Nature Publishing Group**. doi: 10.1038/s41526-017-0019-7.

[4] [4] M. Lalkovicova, 'Neuroprotective agents effective against radiation damage of central nervous system', Sep. 01, 2022, **Wolters Kluwer Medknow Publications**. doi: 10.4103/1673-5374.335137.

[5] J. Restier-Verlet et al., 'Radiation on earth or in space: what does it change?', Apr. 01, 2021, **MDPI AG**. doi: 10.3390/ijms22073739.

[6] D. Beninson et al., 'Annals of the ICRP Published on behalf of the lnternational commission on Radiological Protection I. A-Ilyin, Moscow'.

[7] S. R. Santa Maria, D. B. Marina, S. Massaro Tieze, L. C. Liddell, and S. Bhattacharya, 'BioSentinel: Long-Term Saccharomyces cerevisiae Preservation for a Deep Space Biosensor Mission', **Astrobiology**, vol. 23, no. 6, pp. 617–630, Jun. 2023, doi: 10.1089/ast.2019.2073.

[8] E. Pariset et al., 'DNA Damage Baseline Predicts Resilience to Space Radiation and Radiotherapy', **Cell Rep**, vol. 33, no. 10, Dec. 2020, doi: 10.1016/j.celrep.2020.108434.

[9] J. (ed.) Valentin, 'ICRP Publication 103 - The 2007 Recommendations of the International Commission on Radiological Protection', Elsevier, vol. 37, no. 2–4, pp. 1–332, 2007, doi: 10.1016/j.icrp.2007.10.003.

[10] O. Giacinto et al., 'Cardiovascular Effects of Cosmic Radiation and Microgravity', Jan. 01, 2024, **Multidisciplinary Digital Publishing Institute (MDPI)**. doi: 10.3390/jcm13020520.

[11] 'ICRU Report 51, Quantities and Units in Radiation Protection Dosimetry – ICRU'. Accessed: Apr. 28, 2025. [Online]. Available: https://www.icru.org/report/quantities-and-units-in-radiation-protection-dosimetry-report-51/

[12] N. Suntharalingam, 'Medical Radiation Dosimetry', 1982.

[13] D. Johnson, H. Harold Li, and B. F. Kimler, 'Dosimetry: Was and is an absolute requirement for quality radiation research', **Radiat Res**, vol. 202, no. 2, pp. 102–129, Jul. 2024, doi: 10.1667/RADE-24-00107.1.

[14] G. Bratschitsch et al., 'Radiation Exposure of Patient and Operating Room Personnel by Fluoroscopy and Navigation during Spinal Surgery', **Sci Rep**, vol. 9, no. 1, Dec. 2019, doi: 10.1038/s41598-019-53472-z.

[15] A. F. Cristante, F. Barbieri, A. A. R. da Silva, and J. C. Dellamano, 'Radiation exposure during spine surgery using C-arm fluoroscopy', **Acta Ortop Bras**, vol. 27, no. 1, pp. 46–49, Jan. 2019, doi: 10.1590/1413-785220192701172722.

[16] M. Lassmann and U. Eberlein, 'The relevance of dosimetry in precision medicine', **Journal of Nuclear Medicine**, vol. 59, no. 10, pp. 1494–1499, Oct. 2018, doi: 10.2967/jnumed.117.206649.

[17] A. Amaral, 'Trends in Biological Dosimetry. An Overview', **Brazilian Archives of Biology and Technology**, vol. 62, 2019, doi: 10.1590/1678-4324-2019180573.

[18] M. B. Escalona, T. L. Ryan, and A. S. Balajee, 'Current developments in biodosimetry tools for radiological/nuclear mass casualty incidents', **Environmental Advances**, vol. 9, Oct. 2022, doi: 10.1016/j.envadv.2022.100265.

[19] M. Satyamitra, F. E. Reyes Turcu, N. Pantoja-Galicia, and L. Wathen, 'Challenges and Strategies in the Development of Radiation Biodosimetry Tests for Patient Management', **Radiat Res**, vol. 196, no. 5, pp. 455–467, Nov. 2021, doi: 10.1667/RADE-21-00072.1.

[20] M. Aryankalayil et al., 'Biomarkers for Biodosimetry and Their Role in Predicting Radiation Injury', **Cytogenet Genome Res**, vol. 163, no. 3–4, pp. 103–109, 2023, doi: 10.1159/000531444.

[21] S. Sholom, S. W. S. McKeever, M. B. Escalona, T. L. Ryan, and A. S. Balajee, 'A comparative validation of biodosimetry and physical dosimetry techniques for possible triage applications in emergency dosimetry', **Journal of Radiological Protection**, vol. 42, no. 2, 2022, doi: 10.1088/1361-6498/ac5815.

[22] S. O. ; S. D. N. ; B. O. ; d'Errico, F. Souza, 'Novos materiais dosimétricos para aplicações em física médica', **Revista Brasileira de Física Médica**, vol. 13, no. 1, pp. 24–33, 2019, Accessed: Apr. 28, 2025. [Online]. Available: https://www.rbfm.org.br/rbfm/article/view/509

[23] L. Tauhata, I. Salati, R. Di Prinzio, A. R. Di Prinzio, and R. De Janeiro, 'Radioproteção e dosimetria: Fundamentos. Instituto de Radioproteção e dosimetria. Comissão Nacional de Energia Nuclear'.

[24] H. M. Swartz, 'History of EPR Studies from the H.M. Swartz Laboratories: Part 2—EPR Biodosimetry', Jan. 01, 2022, **Springer.** doi: 10.1007/s00723-021-01451-x.

[25] E. G. Yukihara and S. W. McKeever, 'Optically stimulated luminescence (OSL) dosimetry in medicine.', 2008. doi: 10.1088/0031-9155/53/20/R01.

[26] B. Obryk et al., 'High-Dose TL dosimetry of reactor neutrons', **Radiat Prot Dosimetry**, vol. 180, no. 1–4, pp. 235–239, 2018, doi: 10.1093/RPD/NCX244.

[27] M. Port et al., 'RENEB Inter-Laboratory Comparison 2021: Inter-Assay Comparison of Eight Dosimetry Assays', **Radiat Res**, vol. 199, no. 6, pp. 535–555, Apr. 2023, doi: 10.1667/RADE-22-00207.1.

[28] U. Oestreicher et al., 'RENEB intercomparisons applying the conventional Dicentric Chromosome Assay (DCA)', Int J **Radiat Biol**, vol. 93, no. 1, pp. 20–29, Jan. 2017, doi: 10.1080/09553002.2016.1233370.

[29] S. Sommer, I. Buraczewska, and M. Kruszewski, 'Micronucleus assay: The state of art, and future directions', Feb. 02, 2020, **MDPI AG**. doi: 10.3390/ijms21041534.

[30] J. F. Barquinero et al., 'RENEB biodosimetry intercomparison analyzing translocations by FISH', **Int J Radiat Biol**, vol. 93, no. 1, pp. 30–35, Jan. 2017, doi: 10.1080/09553002.2016.1222092.

[31] V. Raavi, V. Perumal, and S. F.D. Paul, 'Potential application of γ-H2AX as a biodosimetry tool for radiation triage', Jan. 01, 2021, **Elsevier B.V**. doi: 10.1016/j.mrrev.2020.108350.

[32] M. Abend, P. Ostheim, and M. Port, 'Radiation-Induced Gene Expression Changes Used for Biodosimetry and Clinical Outcome Prediction: Challenges and Promises', **Cytogenet Genome Res**, vol. 163, no. 3–4, pp. 223–230, 2023, doi: 10.1159/000530947.

[33] A. S. Balajee, H. C. Turner, and R. C. Wilkins, 'Radiation Biodosimetry: Current Status and Future Initiatives', **Cytogenet Genome Res**, vol. 163, no. 3–4, pp. 85–88, 2023, doi: 10.1159/000535488.

[34] H. M. Swartz, B. B. Williams, and A. B. Flood, 'Overview of the principles and practice of biodosimetry', in **Radiation and Environmental Biophysics**, Springer New York LLC, 2014, pp. 221–232. doi: 10.1007/s00411-014-0522-0.

[35] M. M. P. Lemos-Pinto, M. Cadena, N. Santos, T. S. Fernandes, E. Borges, and A. Amaral, 'A dose-response curve for biodosimetry from a 6 MV electron linear accelerator', **Brazilian Journal of Medical and Biological Research**, vol. 48, no. 10, pp. 908–914, Oct. 2015, doi: 10.1590/1414-431X20154470.

[36] C. Herate and L. Sabatier, 'Retrospective biodosimetry techniques: Focus on cytogenetics assays for individuals exposed to ionizing radiation', **Mutat Res Rev Mutat Res**, vol. 783, 2020, doi: 10.1016/j.mrrev.2019.108287.

[37] M. M. Satyamitra et al., 'Metabolomics in radiation biodosimetry: Current approaches and advances', **Metabolites**, vol. 10, no. 8, pp. 1–28, Aug. 2020, doi: 10.3390/metabo10080328.

[38] T. S. Gnanasekaran, 'Cytogenetic biological dosimetry assays: Recent developments and updates', **Radiat Oncol J**, vol. 39, no. 3, pp. 159–166, 2021, doi: 10.3857/roj.2021.00339.

[39] A. Bertucci, R. C. Wilkins, S. Lachapelle, H. C. Turner, D. J. Brenner, and G. Garty, 'Comparison of Isolated Lymphocyte and Whole Blood-Based CBMN Assays for Radiation Triage', **Cytogenet Genome Res**, vol. 163, no. 3–4, pp. 110–120, Mar. 2024, doi: 10.1159/000533488.

[40] R. Wanotayan et al., 'Quantification of histone H2AX phosphorylation in white blood cells induced by ex vivo gamma irradiation of whole blood by both flow cytometry and foci counting as a dose estimation in rapid triage', **PLoS One**, vol. 17, no. 3 March, Mar. 2022, doi: 10.1371/journal.pone.0265643.

[41] C. H. Anjali and R. Maddaly, 'Applications of Premature Chromosome Condensation technique for genetic analysis', Feb. 01, 2024, **Elsevier Ltd.** doi: 10.1016/j.tiv.2023.105736.

[42] A. Trevisan, O. Ploc, P. Bláha, and C. Scheibler, 'Cancer risks from cosmic radiation exposure in flight: A review'.

[43] F. A. Cucinotta et al., 'Space radiation cancer risks and uncertainties for Mars missions', in **Radiation Research**, Radiation Research Society, 2001, pp. 682–688. doi: 10.1667/0033-7587(2001)156[0682:srcrau]2.0.co;2.

[44] E. Vicente, Z. Vujaskovic, and I. L. Jackson, 'A systematic review of metabolomic and lipidomic candidates for biomarkers in radiation injury', Jun. 01, 2020, **MDPI AG**. doi: 10.3390/metabo10060259.

[45] E. Afshinnekoo et al., 'Fundamental Biological Features of Spaceflight: Advancing the Field to Enable Deep-Space Exploration', Nov. 25, 2020, **Cell Press**. doi: 10.1016/j.cell.2020.10.050.

[46] M. Sproull et al., 'Comparison of Proteomic Expression Profiles after Radiation Exposure across Four Different Species', **Radiat Res**, vol. 197, no. 4, pp. 315–323, Apr. 2022, doi: 10.1667/RADE-21-00182.1.

[47] J. L. Huff et al., 'Galactic cosmic ray simulation at the NASA space radiation laboratory – Progress, challenges and recommendations on mixed-field effects', Feb. 01, 2023, **Elsevier Ltd**. doi: 10.1016/j.lssr.2022.09.001.

[48] L. C. Liddell et al., 'BioSentinel: Validating Sensitivity of Yeast Biosensors to Deep Space Relevant Radiation', **Astrobiology,** vol. 23, no. 6, pp. 648–656, Jun. 2023, doi: 10.1089/ast.2022.0124.

[49] S. A. Abdullaev, S. I. Glukhov, A. I. Gaziev, E. O. Pla, and A. Montoro, 'Radioprotective and Radiomitigative Effects of Melatonin in Tissues with Different Proliferative Activity', 2021, doi: 10.3390/antiox.

[50] M. Ouzzani, H. Hammady, Z. Fedorowicz, and A. Elmagarmid, 'Rayyan-a web and mobile app for systematic reviews', **Syst Rev,** vol. 5, no. 1, Dec. 2016, doi: 10.1186/s13643-016-0384-4.

[51] Z. Munn et al., 'Assessing the risk of bias of quantitative analytical studies: introducing the vision for critical appraisal within JBI systematic reviews', **JBI Evid Synth**, vol. 21, no. 3, pp. 467–471, Dec. 2023, doi: 10.11124/JBIES-22-00224.

[52] F. Tustumi, 'Choosing the most appropriate cut-point for continuous variables', 2022, **Colegio Brasileiro de Cirurgioe**s. doi: 10.1590/0100-6991E-20223346.

[53] E. J. Hall and A. J. Giaccia, **Radiobiology for the radiologist**, 8th ed., vol. 8th ed. Philadelphia, 2019. Accessed: Apr. 22, 2025. [Online]. Available: https://lccn.loc.gov/2017057791

[54] M. L. Riego et al., 'Chromosomal damage, gene expression and alternative transcription in human lymphocytes exposed to mixed ionizing radiation as encountered in space', **Sci Rep**, vol. 14, no. 1, Dec. 2024, doi: 10.1038/s41598-024-62313-7.

[55] D. M. Sridharan, L. C. Chien, F. A. Cucinotta, and J. M. Pluth, 'Comparison of signaling profiles in the low dose range following low and high LET radiation', **Life Sci Space Res (Amst),** vol. 25, pp. 28–41, May 2020, doi: 10.1016/j.lssr.2020.02.002.

[56] M. Engelbrecht et al., 'DNA damage response of haematopoietic stem and progenitor cells to high-LET neutron irradiation', Sci Rep, vol. 11, no. 1, Dec. 2021, doi: 10.1038/s41598-021-00229-2.

[57] J. C. Nwanaji-Enwerem, P. Boileau, J. M. Galazka, and A. Cardenas, 'In vitro relationships of galactic cosmic radiation and epigenetic clocks in human bronchial epithelial cells', **Environ Mol Mutagen**, vol. 63, no. 4, pp. 184–189, Apr. 2022, doi: 10.1002/em.22483.

[58] A. Kowalska, E. Nasonova, K. Czerski, P. Kutsalo, W. Pereira, and E. Krasavin, 'Production and distribution of chromosome aberrations in human lymphocytes by particle beams with different LET', **Radiat Environ Biophys**, vol. 58, no. 1, pp. 99–108, Mar. 2019, doi: 10.1007/s00411-018-0771-4.

[59] W. E. Radstake et al., 'Radiation-induced DNA double-strand breaks in cortisol exposed fibroblasts as quantified with the novel foci-integrated damage complexity score (FIDCS)', **Sci Rep**, vol. 14, no. 1, Dec. 2024, doi: 10.1038/s41598-024-60912-y.

[60] 'Radiological protection-Performance criteria for laboratories using the cytokinesis-block micronucleus (CBMN) assay in peripheral blood lymphocytes for biological dosimetry International Standard, 2024. [Online]. Available: www.iso.org

[61] G. Dietze et al., 'ICRP publication 123: Assessment of radiation exposure of astronauts in space', **Ann ICRP**, vol. 42, no. 4, pp. 1–339, 2013, doi: 10.1016/j.icrp.2013.05.004.

[62] ISO (International Organization for Standardization), 'ISO. Radiological protection — Performance criteria for service laboratories performing biological dosimetry by cytogenetics — Dicentric assay. ISO 19238:2023(E)', Geneva, 2023.

[63] R. M'Kacher et al., 'High Resolution and Automatable Cytogenetic Biodosimetry Using In Situ Telomere and Centromere Hybridization for the Accurate Detection of DNA Damage: An Overview', Mar. 01, 2023, **Multidisciplinary Digital Publishing Institute (MDPI)**. doi: 10.3390/ijms24065699.

LICENSE