

Implementation of the Chromosomal Aberration Technique for Monitoring Occupational Exposure to Genotoxic Agents in INCA's Toxicology Laboratory

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Implementação da Técnica de Aberrações Cromossômicas para o Monitoramento da Exposição Ocupacional a Agentes Genotóxicos no Laboratório de Toxicologia do INCA

Implementación de la Técnica de Aberraciones Cromosómicas para el Monitoreo de la Exposición Ocupacional a Agentes Genotóxicos en el Laboratorio de Toxicología del INCA

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ABSTRACT

Introduction: Occupational exposure to chemical agents is associated with genetic damage and an increased risk of diseases, including cancer. Biomonitoring enables early detection of genetic alterations and supports preventive measures. **Objective:** To implement and standardize the chromosomal aberration test at the Laboratory of Occupational and Environmental Toxicology and Cancer Surveillance from the Prevention and Surveillance Coordination of the National Cancer Institute, aiming its application in biomonitoring projects for workers exposed to genotoxic agents. **Method:** A standard operational protocol was developed based on the literature, international guidelines, and technical expertise. The pilot study included a negative control (healthy individuals) and a positive control (samples exposed *in vitro* to methyl methanesulfonate at two concentrations). Slides were prepared and stained with Giemsa, and when necessary, G-banding, analyzing 300 metaphases per sample using optical microscopy and imaging capture systems. Structural and numerical chromosomal alterations were assessed. **Results:** The analysis warranted the implementation of the technique and standardization of the protocol, demonstrating its effectiveness in detecting genetic alterations under controlled experimental conditions. **Conclusion:** The protocol is suitable for future biomonitoring projects, providing a reliable tool for early evaluation of genetic damage and prevention of occupational risks.

Key words: Neoplasms; Occupational Exposure/prevention & control; Chromosomal Aberrations; Biological Monitoring; Genotoxicity.

RESUMO

Introdução: A exposição ocupacional a agentes químicos está associada a danos genéticos e ao aumento do risco de doenças, incluindo câncer. O biomonitoramento permite detecção precoce de alterações genéticas e apoio a medidas preventivas. **Objetivo:** Implementar e padronizar o teste de aberrações cromossômicas no Laboratório de Toxicologia Ocupacional, Ambiental e Vigilância do Câncer da Coordenação de Prevenção e Vigilância do Instituto Nacional de Câncer, visando à sua aplicação em projetos de biomonitoramento de trabalhadores expostos a agentes genotóxicos. **Método:** Foi desenvolvido um protocolo operacional padrão baseado em literatura, diretrizes internacionais e experiência técnica. O estudo-piloto incluiu controle negativo (indivíduos saudáveis) e controle positivo (amostras expostas *in vitro* a metil metanossulfonato em duas concentrações). As lâminas foram preparadas e coradas com Giemsa, e quando necessário o bandejamento G, analisando-se 300 metafases por amostra com microscopia óptica e sistemas de captura de imagem. Alterações cromossômicas estruturais e numéricas foram avaliadas. **Resultados:** A análise permitiu implementar a técnica e padronizar o protocolo, demonstrando sua eficácia para identificar alterações genéticas em condições experimentais controladas. **Conclusão:** O protocolo é adequado para futuros projetos de biomonitoramento, fornecendo ferramenta confiável para avaliação precoce de danos genéticos e prevenção de riscos ocupacionais.

Palavras-chave: Neoplasias; Exposição Ocupacional/prevenção & controle; Aberrações Cromossômicas; Monitoramento Biológico; Genotoxicidade.

RESUMEN

Introducción: La exposición ocupacional a agentes químicos se asocia con daños genéticos y un mayor riesgo de enfermedades, incluido el cáncer. El biomonitoreo permite la detección temprana de alteraciones genéticas y el apoyo a medidas preventivas. **Objetivo:** Implementar y estandarizar la prueba de aberraciones cromosómicas en el Laboratorio de Toxicología Ocupacional, Ambiental y Vigilancia del Cáncer, de la Coordinación de Prevención y Vigilancia del Instituto Nacional del Cáncer, con el fin de aplicarla en proyectos de biomonitoreo de trabajadores expuestos a agentes genotóxicos. **Método:** Se desarrolló un protocolo operativo estándar basado en la literatura, directrices internacionales y experiencia técnica. El estudio piloto incluyó un control negativo (individuos sanos) y un control positivo (muestras expuestas *in vitro* a metanosulfonato de metilo en dos concentraciones). Se prepararon las láminas y se tiñeron con Giemsa y, cuando fue necesario, con bandeo G, analizándose 300 metafases por muestra mediante microscopía óptica y sistemas de captura de imágenes. Se evaluaron alteraciones cromosómicas estructurales y numéricas. **Resultados:** El análisis permitió implementar la técnica y estandarizar el protocolo, demostrando su eficacia para identificar alteraciones genéticas en condiciones experimentales controladas. **Conclusión:** El protocolo es adecuado para futuros proyectos de biomonitoreo, proporcionando una herramienta confiable para la evaluación temprana de daños genéticos y prevención de riesgos ocupacionales.

Palabras clave: Neoplasias; Exposición Laboral/prevenición y control; Aberraciones Cromosómicas; Monitoreo Biológico; Genotoxicidad.

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INTRODUCTION

The damaging effects of chemical agents on human health have been widely studied since the last century, a reality present specially in the occupational context where exposure to mutagenic and carcinogenic substances is a relevant problem of public health and for the worker. Scientific evidences indicate that occupational exposure to these agents is associated with increased risk of development of several diseases such as neurologic disorders, reproductive alterations and cancer¹. The toxicity of these agents depends on a series of factors, including their inherent chemical properties as well as chemical interactions due to the presence of other agents that are part of the formulation of these products, including stabilizers, solvents, impurities, additives and pesticides. These compounds can cause genetic damages, which is highly relevant for public health, especially for the health of the worker due to the already established relation between genotoxicity and development of cancer.

In this context, biomonitoring of individuals exposed stand out as an essential evaluation tool of health surveillance for early detection of adverse biological effects and support to the implementation of preventive and corrective measures in the working environment as a result of the exposure to chemical substances^{2,3}.

The genotoxic potential of chemical substances can be evaluated through a variety of assays related to genetic toxicology such as the micronucleus assay, the comet assay and chromosomal aberration test that can detect DNA damages⁴.

One of the biological consequences of human exposure to chemical agents is the induction to chromosomal alterations that are observed through the use of a methodology called chromosomal aberrations. The study of chromosomal aberrations is a classic cytogenetic evaluation widely validated and accepted internationally that consists of the analysis of the chromosomes obtained by culture of human lymphocytes *in vitro*. This technique is considered an important biomarker of early effects of genotoxic carcinogens in approaches of environmental and occupational exposure. It is essential to understand the alterations that can occur in the genetic material and its complexity lies in the necessity of proper preparation of the samples, specific staining of the chromosomes and careful interpretation of the results.

One of the advantages of evaluating human lymphocytes is the fact that they circulate throughout the entire organism⁵, which exposes them to any mutagenic agent regardless of their route of introduction in the organism, orally, by inhalation and skin.

Chromosomal aberrations are divided in two groups: numeric alterations and structural alterations. Numeric alterations happen when the chromosomes of a cell change, with losses or gains, different from the diploid number ($2n=46$ chromosomes). Structural alterations refer to changes that affect the structure of the chromosomes and are called chromosomal alterations when occur simultaneously in two chromatids or chromatid alterations involving only one chromatid.

Evidences indicate a positive correlation between the increase of the frequency of chromosomal aberrations in lymphocytes and the risk of developing diseases such as neoplasms⁵, which reinforces the relevance of the use of these biomarkers in programs of occupational biomonitoring.

The laboratory of occupational, environmental toxicology and cancer surveillance (LabTox) develops research projects targeted to the worker's health to evaluate the occupational exposure to potentially mutagenic and carcinogenic agents. In this context, the implementation of the technique of chromosomal aberrations is an important expansion of the methodologies of genetic toxicology already adopted in the laboratory to evaluate DNA damages, allowing complementary analyzes and favoring robust interpretation of the results, further to strengthening methodological precision and analytical reproducibility in future biomonitoring research projects developed at the laboratory.

In face of the importance of this biomarker, the objective of the present study is not only implement the test of chromosomal aberrations in LabTox to apply in biomonitoring of workers exposed to mutagenic, carcinogenic and/or potentially carcinogenic agents including the proposal to evaluate potential health risks in clinical trials approved by INCA's Ethics Committee but also to integrate it to other methodologies already implemented in the lab and expanding the scope and robustness of the results obtained.

METHOD

A comprehensive bibliographic review aligned with the accumulated technical experience of the professional in charge of the execution and interpretation of the test of chromosomal aberrations was conducted for the implementation of the technique and elaboration of the standard operational protocol. The procedures adopted in all the phases were driven by international guidelines (OECD)⁶, and in compliance with the Clinical Laboratory Accreditation Program (PALC) Standard⁷, ensuring conformity with the recommendations of the scientific literature and good laboratory clinical practices consolidated in the lab routine.

The standard operational protocol was elaborated and describes in detail each step of the operational and experimental routine adopted in the test.

Two groups were evaluated for the pilot-study: (1) negative control group containing peripheral blood samples of clinically healthy volunteers without history of occupational exposure to chemical agents; (2) positive control group formed by peripheral blood samples of volunteers in the same conditions of the negative control group with the addition of the mutagenic agent Methyl Methanesulfonate (MMS – Sigma®), at different concentrations to the cell culture to induce *in vitro* chromosomal alterations.

The peripheral blood samples were collected in tubes with heparin and stored in 2 to 4°C fridges until processing. The cultures of lymphocytes were prepared in 15 ml Falcon tubes containing 5 ml of mean PB-MAX (Gibco®), a market commercial mean for karyotype formed by RPMI, enriched with fetal bovine serum (FBS), supplemented with phytohemagglutinin (PHA) and 1.0 ml of total blood in CO₂ incubator (5%) at 37°C.

The cultures of the samples of the negative control group were represented by two volunteers and considered two distinct sample incubation times: 48 and 72 hours. For the positive control group, the cultures were represented by four volunteers with the same time of incubation. The mutagenic agent MMS was added to the cultures after 24 hours (cultures of 48 h) and after 48 hours (cultures of 72 h), ensuring exposure of 24 hours to the agent. Two distinct concentrations were tested: 80 µM and 160 µM, according to guidelines of OECD and specialized literature^{8,9} in order to identify the experimental interval that warranted the optimal cell yield and select the condition that presented the expected cytogenetic effect for the definition of the assay protocol.

After the incubation period, 48 or 72 hours, more or less one hour before or after, 0.165 µg/ml of colchicine (Sigma®) was added to all the samples to disrupt the formation of the mitotic spindle and arresting cell division in metaphase. Next, the cultures were incubated again for more one hour at 37 °C.

At the end of this period, the tubes were centrifuged at 1,500 rpm for 10 minutes, the supernatant was removed and 10 ml of hypotonic solution were added (KCl 0.075 M), homogenized and kept at 37 °C in water bath for 30 minutes.

After this period, the tubes were centrifuged once more, the supernatant was removed and the fixation phase started when 10 ml of recently prepared and refrigerated fixator were added (Carnoy's solution; methanol and acetic acid, Merck, 3:1), with two more successive stages of

lavage with fixator, leaving the pellet cleaner when 5 ml of fixator were added in each one followed by centrifugation.

After the lavage and last centrifugation, approximately 0.5mL of the fixator was kept with the cellular sediment (pellet), the suspension was homogenized for the preparation of the slides. These were prepared using the cell suspension dripping technique and placed on a hot plate at 50 °C for 10 to 15 minutes or kept at room temperature for drying. In average, two to four slides per participant were prepared.

The slides were submerged in a 5% Giemsa solution diluted in phosphate-buffered saline (PBS) inside a Coplin jar for 5 minutes, rinsed under running tap water and the slides were left to air-dry at room temperature.

Figure 1 shows all the required steps for the chromosomal aberrations test.

The entire technique was based on the method originally developed¹⁰, modified and adapted. Before the definitive standardization of the standard operational protocol, tests with negative and positive controls were conducted followed by adjustments to improve the quality and quantity of the cells. These adjustments included: (1) increase of the blood volume from 0.5ml to 1.0ml per culture to raise the leucocyte density; (2) optimization of the mitotic block with colchicine through adjustment of the concentration to 0.165µg/ml after different tests (tested interval of 0.033 to 0.165µg/ml), with exposure time to 60 minutes (tested between 30 and 60 minutes), ensuring a satisfactory mitotic index without compromising the chromosomal morphology; (3) standardization of the hypotonic treatment (KCl 0.075 M) for 30 minutes (tested from 20 to 30 minutes), favoring chromosomal dispersion; (4) adjustment of the speed of centrifugation to 1,500 rpm (tested between 1,500 and 1,800rpm) to ensure the integrity of the pellet. These adaptations were critical for protocol adequacy, resulting in better preservation of the structural integrity of the chromosomes, essential for the efficacy of detecting genotoxic damages.

The slides were analyzed in an optical microscope to evaluate the quality of the material by screening metaphases frequency and chromosomes morphology in addition to verifying the concentration of the cell suspension (Figure 2). The mitotic index was evaluated through the count of the number of metaphases found in 1,000 lymphocytes observed.

In addition to conventional staining, the G-banding technique was also tested according to the original method, with modifications¹¹. The technique consists of treating the chromosomes with trypsin whose function is to denature the chromosomes proteins, partially digesting the chromosomal regions and presenting two band patterns: heterochromatic regions, rich in adenine and

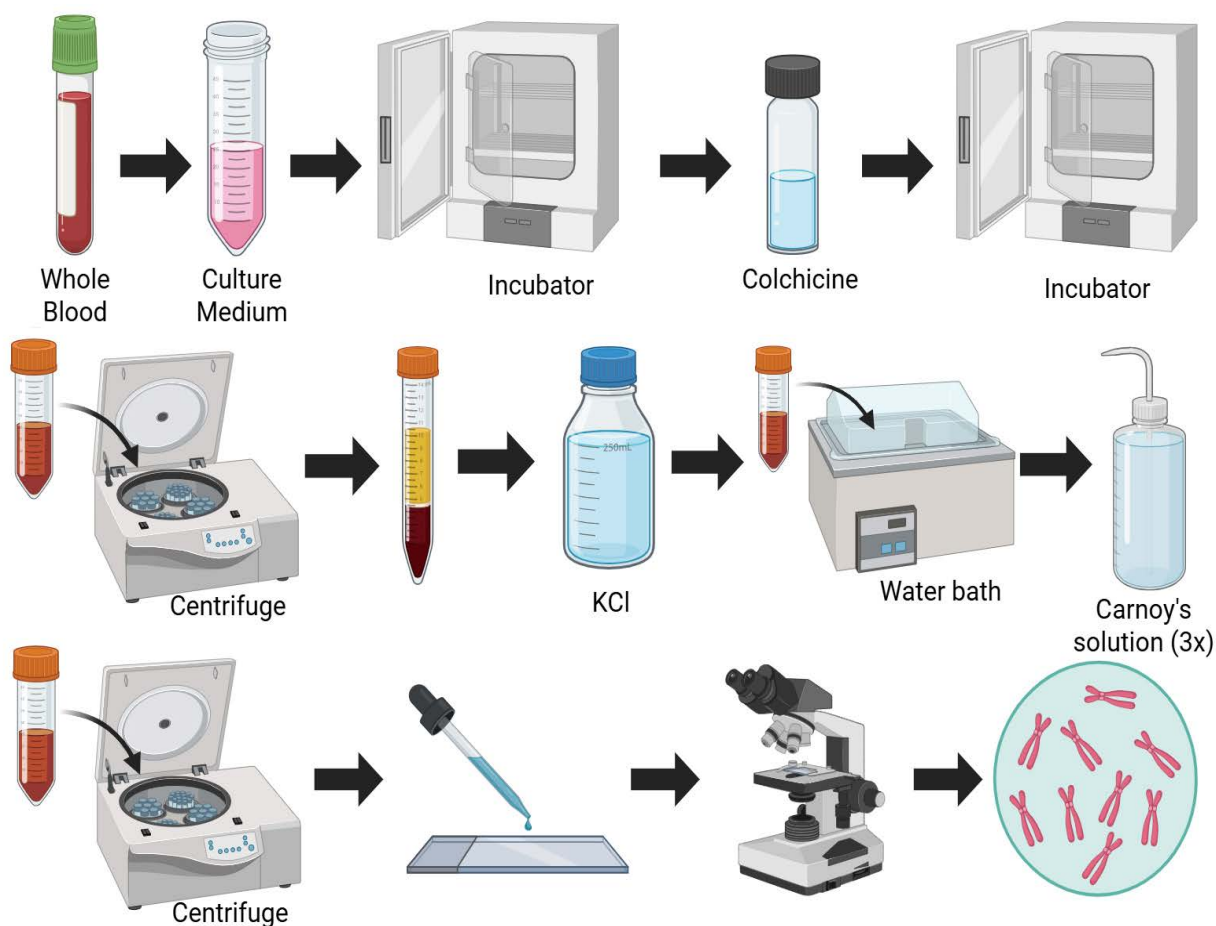


Figure 1. Flowchart of all the steps of the chromosomal aberrations test
Note: Image created with the method of the software BioRender.

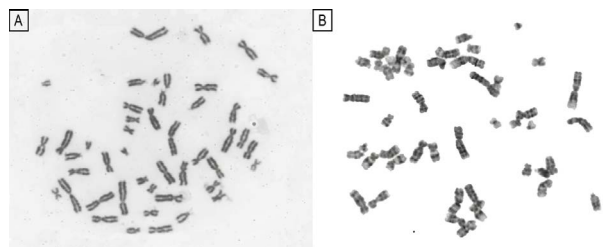


Figure 2. Human chromosomes unchanged in metaphase (lymphocytes culture): (A) Chromosomes in conventional staining; (B) Chromosomes G-Banding

Note: Images obtained by Metafer analysis platform.

thymine (AT) and producing dark bands and euchromatic regions, rich in guanine and cytosine (GC), absorb less Giemsa and create light bands (Figure 2B). The slides were submitted to ageing process for periods between 2 and 4 hours in 50 °C hot plate, 24 hours in 37 °C incubator or 72 hours at room temperature. The ideal conditions for banding can vary significantly due to several factors as room temperature and humidity, culture time, quality of the slides, among others.

For banding, a trypsin solution (Sigma®) was prepared by dissolving 0.10 g in 100 ml of Dulbecco's

buffer and heated at 37 °C. The slides were submitted to trypsinization in Coplin jar for 5-60 second periods with exposure time proportional to the period and condition of ageing. Next, they were bathed in physiologic solution and finally stained with 5% Giemsa for 5 minutes, rinsed under running tap water and left to dry at room temperature.

The slides were initially analyzed in optical microscope, the mitotic index was evaluated and 300 metaphases were counted in conventional staining (according to recommendation of OECD), considering only cells with 44 to 46 chromosomes or more. The samples that needed to be evaluated by karyotype to confirm constitutional alterations were submitted to G-banding technique that ensured better analysis of the alterations and identification of the chromosomes involved. When necessary, 20 metaphases were karyotyped per individual according to quality norms and the result was described in compliance with the International System for Human Cytogenomic Nomenclature (ISCN)¹².

The identification of the karyotype is done after G-banding exclusively, which allows a thorough analysis

with the distinction of pairs of autosomal and sexual chromosomes, in addition to the detection of more subtle alterations as deletions, inversions, duplications among others. All the double-checked analyzes were conducted by two or more trained experts and revised whenever necessary according to recommendations of norms of quality.

The cells with chromosomal aberrations were logged in a spreadsheet and classified per type and quantity of alterations such as translocations, breaks and chromatid failures among others. This categorization favored the statistical analysis.

To optimize the analyzes and construction of karyotypes, in addition to manual counting in microscope, the slides were also evaluated through an analysis system integrated to imaging capture, equipped with edition tools. The semiautomated system Metafer¹³ (software of MetaSystems) and the manual system GeneALL¹⁴ (OutSet) were utilized.

The pilot-study was conducted with a six female volunteers of the lab staff in a convenience sample, aged between 21-31 years, who were informed that the blood samples would be used exclusively for internal control and standardization of the methodology. These samples were randomly distributed in groups of two volunteers for each experimental condition in order to optimize the variables and standardization of the protocol to be utilized in the laboratory. The volunteers were asked about the exposure of ionizing radiation (X-ray, computed tomography, bone densitometry, scintigraphy and mammogram) because they are confounding variables for genotoxic effect to be checked in the assay.

The tests conducted allowed the implementation and standardization of the chromosomal aberrations technique to be utilized in clinical trials evaluating the occupational exposure to chemical agents in the working environment. INCA's Ethics Committee approved the study, report number 2,983,493 (CAAE (submission for ethical review): 64799217.3.0000.5274), connected to the project "*Investigação dos efeitos tóxicos sobre a saúde de trabalhadores do município de Casimiro de Abreu (RJ) expostos a agrotóxicos*", which included the chromosomal aberrations technique in compliance with Directive 466/12¹⁵ of the National Health Council for studies with human beings.

RESULTS

The microscopic analysis of the samples led to the classification of the metaphases and chromosomes based on parameters described in the cytogenetic literature such as the number of metaphases obtained, integrity and chromosome morphology, degree of chromosome spreading and overlapping chromosomes (Chart 1).

Chart 1. Classification of the conditions of culture post microscopic evaluation

Evaluation	Classification	Criterion
Quantitative	Good	100 metaphases per slide
Quantitative	Satisfactory	Between 50 and 100 metaphases per slide
Quantitative	Low	Less than 50 metaphases per slide
Quantitative	Insufficient or TAM	Few or total absence of metaphases
Qualitative	Good	Intact chromosomes, clear morphology, good spread and minimal overlapping
Qualitative	Satisfactory	Little condensed chromosomes or much spread, moderate overlapping and moderate cytoplasm
Qualitative	Poor	Agglomerated chromosomes, excess condensation and excess of cytoplasm

Caption: TAM = Total Absence of Metaphases.

The different conditions of culture utilized for the optimization and standardization of the technique of chromosomal aberrations are detailed in Table 1. The samples were distributed randomly in groups of two volunteers for each experimental condition, considering variations of culture time, blood volume, concentration of MMS, concentration and time of exposure of colchicine in addition to the hypotonization with KCL.

The results show that 48-hour cultures compared to 72-hour cultures present low yield in the different experimental conditions both for positive or even negative controls as can be seen in Table 1.

While evaluating specifically the cultures treated with MMS utilized as positive control, the 48-hour culture time did not present satisfactory results, showing low mitotic index and metaphases with low quality chromosomes. For this reason, this time of culture was rejected for the positive controls, being kept only the 72-hour with determined concentrations of MMS. In counterpart, the cultures not treated with MMS (negative controls) presented satisfactory results for both times of culture (48 h and 72 h). Thus, the two periods were established as standard. All the cultures were identified as C1 for the 48-hour time whereas they were identified as C2 for the 72-hour time culture.

The microscopic evaluation of the metaphase chromosomes of samples treated with positive control MMS showed the presence of the structural alterations

Table 1. Characterization of the different variables utilized

Culture time (h)	Blood volume (ml)	Concentration MMS (uM)	Concentration colchicine (ug/ml)	Time of colchicine (min)	Time of KCl	Quantity	Quality
48	0.5	0	0.066	30	20	Poor	Poor
48	0.5	0	0.066	30	20	Poor	Poor
48	0.5	0	0.066	60	20	Poor	Poor
48	0.5	0	0.066	60	20	Poor	Poor
48	1	0	0.165	60	30	Poor	Satisfactory
48	1	0	0.165	60	30	Satisfactory	Satisfactory
48	1	0	0.165	60	30	Satisfactory	Satisfactory
48	1	0	0.165	60	30	Good	Satisfactory
48	1	160	0.165	60	30	Poor	Satisfactory
48	1	160	0.165	60	30	Good	Satisfactory
72	0.5	0	0.033	60	20	Good	Satisfactory
72	0.5	0	0.033	60	20	Poor	Satisfactory
72	0.5	0	0.066	60	20	Good	Satisfactory
72	0.5	0	0.066	60	20	Good	Satisfactory
72	0.5	0	0.165	60	20	Good	Satisfactory
72	0.5	0	0.165	60	20	Good	Satisfactory
72	1	0	0.165	60	30	Good	Satisfactory
72	1	0	0.165	60	30	Good	Good
72	1	80	0.165	60	30	Good	Good
72	1	80	0.165	60	30	Good	Satisfactory
72	1	160	0.165	60	30	Good	Satisfactory
72	1	160	0.165	60	30	Good	Good

Captions: MMS = Methyl Methanesulfonate; KCl = Potassium chloride.

such as: chromatid break (5.5%, n=2), dicentric chromosome (5.5%, n=2), acentric fragment (16.6%, n=6), chromosomal minutes (25%, n=9), centromeric separation (11.1%, n=4), translocation (8.3%, n=3), chromatid gap (25%, n=9), quadricentric chromosome (2.7%, n=1), proving the sensitiveness of the method of detection of induced damages (Figure 3).

DISCUSSION

The implementation of the Chromosomal Aberration Test in LabTox allowed the consolidation of a standardized protocol based on OECD⁶ guidelines, and in national norms of laboratory quality⁷, favoring analytical reproducibility, while also enabling comparison with studies conducted at different research centers.

The pilot-study revealed technical sensitiveness to detect induced aberrations, demonstrated by the presence of structural chromosomal aberrations like centromeric separation, chromatid gap, chromatid break,

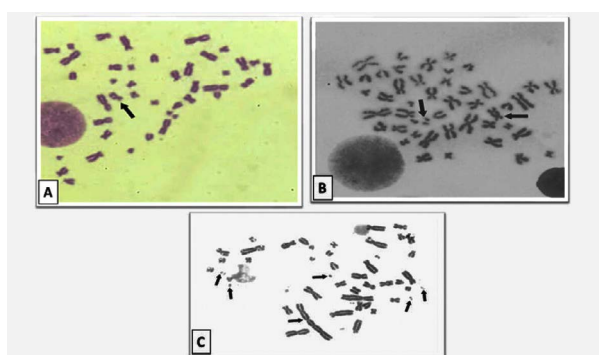


Figure 3. Image of altered metaphases (arrows) seen in positive control samples: (A) chromatid break; (B) dicentric chromosomes and acentric fragment; (C) quadricentric chromosome with fragments acentric and minutes

Note: Images obtained from the System of Capture and Analysis GeneAll

dicentric chromosome with acentric fragment, a possible quadricentric chromosome and minutes in the cultures treated with MMS, confirming the adequacy as tool of positive control applied to the standardization of the technique. These findings corroborate the literature that

describes MMS as an inductive agent of chromosomal damages^{6,16}.

The results demonstrate that the culture time directly influenced the quality of the preparations, especially in cultures treated with MMS. The 48-hour period is often insufficient to ensure appropriate cellular progression, possibly because many cells were still in the first mitosis, resulting in a lower number of metaphases and compromising the quality of the analysis, in addition that the exposure to MMS interferes in the dynamics of the cellular cycle, reducing the frequency of cells with this time of division. In counterpart, the 72-hour culture was more adequate for the cultures, because it represents one and a half cycle of division in lymphocytes that present asynchronous cell division.

The evaluation of different culture times contributed to the optimization of the protocol, the 72-hour period presented the best cytogenic performance and was defined as positive controls standard. The cultures that kept the times of 48 and 72 hours for negative controls expanded the methodological flexibility and applicability of the technique to distinct experimental designs. The incorporation of the G-banding technique as complementary method helped to characterize the alterations and confirmation of constitutional alterations, increasing the interpretative robustness of the results.

The study limitations are the predominantly methodologic aspects of a pilot study. The reduced sample size limited the execution of statistical analyzes and generalization of the findings and more robust experimental comparisons, especially because of the small number of metaphases observed in the cultures treated with MMS in the 48-hour period. Consequently, the interpretation of the data was based on quantitative descriptive criteria like the mitotic index and qualitative such as the integrity of the metaphases and the morphology of the chromosomes. In this stage of the implementation, the negative controls were utilized exclusively as qualitative reference for the monitoring of the feasibility of the cultures. However, these limitations do not invalidate the study, since the results achieved were sufficient to implement the technique and consolidate a standardized and reproducible protocol.

In the context of health surveillance, the consolidation of the chromosomal aberrations test in LabTox reinforces the genetic biomonitoring as a predictive tool, given the association between the frequency of chromosomal aberrations and the risk of development of neoplasms^{17,18}. The elaboration of a standard operational protocol and the implementation of the technique not only harmonizes the in-house methodology but also qualify LabTox for future applications in occupational exposed populations. The

integration of this assay with other biomarkers and analysis of individual variables can expand the understanding of risks in collective health.

These results corroborate the literature that emphasizes the relevance of the chromosomal aberrations test as a classic biomarker in the evaluation of the exposure to genotoxic agents in studies of human biomonitoring. The appropriate standardization of the experimental conditions, including culture time and use of proper controls, can ensure the comparability of the results among different laboratories.

Therefore, the implementation of the chromosomal aberrations test in LabTox, allied to the elaboration of a standard operational protocol is a significant advance to the methodological harmonization and generation of scientific data, expanding its potential of application in programs of genetic biomonitoring and contributing for the early evaluation of occupational hazards and development of prevention strategies and protection of the worker's health.

CONCLUSION

The consolidation of the implementation of the chromosomal aberrations test in LabTox together with the development of a standard operational protocol contributed to the standardization and reproducibility of the technique, strengthening the reliability of the results achieved to favor the methodological harmonization with other research centers because it is a tool with potential to directly contribute for the generation of relevant scientific data that will be able to support interventions targeted to the worker's health. In addition, the implementation of the technique allows the integration of this biomarker with the programs of genetic biomonitoring as a promising tool to guide preventive measures and evaluation of the efficacy of actions of control in populations occupationally exposed to genotoxic agents.

Although the limitations of the study related to the pilot feature and reduced sample size, the results obtained allowed the standardization of the protocol, showing that the technique is able to ensure robustness in future analyzes.

The study shows the importance of incorporating one more technique of evaluation of the genotoxic damage that, associated with other methodologies already implemented, potentializes LabTox in the interface between applied research, toxicology and the worker's health.

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CONTRIBUTIONS

Ana Paula Castilho contributed to the conception and design of the study, analysis and interpretation of the data, writing and critical review. Katia Soares da Poça contributed to the analysis and interpretation of the data and critical review. Maria Theresa de Souza Accioly Martins contributed to the study design and critical review. Márcia Sarpa de Campos Mello contributed to the writing and critical review. All the authors approved the final version for publication.

DECLARATION OF CONFLICT OF INTEREST

There is no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

All the content underlying the text is available upon request to the corresponding author.

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