CHRONOLOGY OF ACHIEVEMENTS

YEAR	ACHIEVEMENT	REFERENCE
1997	Founding of HUMN project at 7th ICEM in Toulouse, France. http://www.iaemgs.org/Historical.asp	[1]
1997	Analysis of results from children exposed to the Chernobyl catastrophe showed that the lymphocyte CBMN assay could detect the genotoxic effects of radionuclide contamination and that increased in MN frequency was discernible both in mononucleated and binucleated cells	[2]
2001	A proposal was made that inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes which led to the concept of the cytokinesis-block micronucleus "cytome" (CBMNcyt) assay. Subsequently measurement of nuclear buds and nucleoplasmic bridges were added to the cytome concept.	[3,4]
2001	Collated International database of base-line MN frequency in binucleated human lymphocytes using the cytokinesis-block micronucleus assay and evaluated the effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei.	[5]
2003	Completed international slide scoring exercise to determine Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes.	[6]
2003	Published detailed description of the scoring criteria for identifying micronuclei, nucleoplasmic bridges and nuclear buds in mononucleated or binucleated cells, necrotic cells and apoptotic cells in the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures.	[7]
2003	The effect of smoking habit on the frequency of micronuclei in human lymphocyte was determined and published.	[8]
2005	Analysis of data from a dietary intake and micronucleus index survey in South Australia revealed that low intake of calcium, folate, nicotinic acid, vitamin E, retinol, beta-carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability.	[9]
2006	The effects of GSTM1 and GSTT1 polymorphisms on micronucleus frequencies in human lymphocytes were investigated and published.	[10]
2007	A prospective study of 6718 subjects from of 10 countries, linking base-line MN with cancer incidence data, revealed for the first time that an increased MN frequency in peripheral blood lymphocytes predicts the risk of cancer in humans.	[11]
2007	A new HUMN project was developed and communicated, focusing on the standardisation and harmonisation of the micronucleus assay in human buccal cells as well as the collation and analysis of buccal MN frequency in diverse human populations.	[12]
2007	A detailed protocol for the cytokinesis-block micronucleus cytome assay in peripheral blood lymphocytes was published in Nature Protocols.	[13]
2008	Another paper investigated and reported on the effects of carriage of the hOGG1(326), XRCC1(399) and XRCC3(241) polymorphisms on micronucleus frequencies in human lymphocytes in vivo.	[14]
2008	The HUMN project perspective on current status and knowledge gaps of the micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage was published. This report also included contributions from a workshop on the same topic held in Turkey (see below)	[15]
2008	A buccal micronucleus assay workshop was organized by the HUMN project- in Antalya, Turkey in 2007 to develop a strategy to improve the method and validate the assay. The outcomes of the workshop were published.	[16]
2009	A detailed protocol of the buccal micronucleus cytome assay was published in Nature Protocols	[17]
2009	Data from 43 laboratories relating to their methodology of the buccal micronucleus assay and type of epidemiological data that are routinely collected were collected and analysed. This revealed a potential base of 15,103 subjects for pooled analyses which contributed to (i) identifying technical and epidemiological key variables that impact on buccal MN frequency in human populations, (ii) drive the design of future intra- and interlaboratory validation studies	[18]

	and (iii) determine the role of MN frequency and other biomarkers, in monitoring genomic damage and predicting cancer and other degenerative diseases.	
2010	Statistical and epidemiological issues when performing human population studies with the exfoliated buccal micronucleus assay were identified by analysis of 63 studies and recommendations for improvement were recommended: inclusion of power analysis in the study design, control for confounding factors, justification for choice of the statistical model, and the number of cells to be scored.	[19]
2010	A paper was published on the application of the lymphocyte cytokinesis-block micronucleus cytome assay in biodosimetry of ionising radiation exposure.	[20]
2011	In 2011 the HUMN project coordinated and published several review papers on the use of micronucleus assays in in vivo human studies in an invited special issue to celebrate the 25th anniversary of the founding of the journal Mutagenesis. The papers reported progress and new research opportunities on a wide range of projects: • Transcriptomic network analysis of MN-related genes [21] • MN as biological dosimeter of ionising radiation exposure [22] • Effect of genetic polymorphisms on MN frequency [23] • Effect of age, gender, diet and lifestyle on MN index [24] • MN in neonates and children [25] • Association of MN with infertility and pregnancy complications [26] • Modulation of MN frequency by nutritional intervention [27] • MN frequency and incidence of cancer, diabetes, cardiovascular and neurodegenerative diseases [28, 29, 30] • Molecular mechanisms of MN, NPB and NBUD formation [31] • Use of nasal cells in human MN studies [32] • Study design and statistical analysis of human MN studies [33] • Past, present and future of the HUMN and HUMNxl projects [34] • Reviews on automated MN scoring systems [35-38].	[21-38]
2011	The first comprehensive analysis of the data from the HUman MicroNucleus project on eXfoLiated buccal cells (HUMN(XL) was completed and the impact of lifestyle, host factors, occupational exposures, health status, and assay protocol on MN frequency in human buccal cells was elucidated and published.	[39]
2013	Completed review of validation, quality control and prospects for further development of automated micronucleus assays using image cytometry systems.	[40]
2013	Published results obtained using the HUMN database on base-line MN frequency to estimate the lowest dose of ionizing radiation exposure that is detectable using the lymphocyte cytokinesis-block micronucleus assay.	[41]
2014	A paper was published on the development and validation of a new method for automation of the cytokinesis-block micronucleus cytome assay using laser scanning cytometry and its potential application in radiation biodosimetry.	[42]
2014	A review was reported on the micronucleus assay with urine derived cells (UDC) and its application in human studies investigating genotoxin exposure and bladder cancer risk.	[43]
2014	A commentary was published on current knowledge gaps and misconceptions on the use of the lymphocyte cytokinesis-block micronucleus assay for in vivo biomonitoring of human exposure to genotoxic chemicals. A roadmap on how to further improve knowledge on the proper application of this assay to study chemical induction of chromosomal damage was proposed.	[44]
2015	Meta-analysis and systematic review of human studies using the buccal micronucleus assay was completed and published. This study showed that this biomarker may be useful in the clinical setting for pre-screening of oral cancer and in the follow up of precancerous oral lesions and may also reflect chromosomal instability of other organs.	[45]
2016	In 2016 the HUMN project coordinated and published several review papers in a special issue on the use of the lymphocyte cytokinesis-block micronucleus (CBMN) assay to measure DNA damage induced in vivo in humans exposed to chemical genotoxins. The papers reported progress and new research opportunities on a wide range of projects relating to this topic including, current knowledge of molecular mechanisms, systematic reviews and meta-analyses	[46-61]

	of epidemiological studies and a synthesis of the data from all the reviewed studies. The topics of the papers were as follows: • Molecular mechanisms by which in vivo exposure to chemical genotoxins leads to MN formation in blood lymphocytes in vivo or ex vivo [46] • A review of DNA damage studies in medical workers exposed to anaesthetic gases assessed by the lymphocyte CBMN assay [47]. • A systematic review of studies on the association between occupational exposure to cytostatic/anti-neoplastic drugs and DNA damage measured by the lymphocyte CBMN assay [48] • Studies on the effect of formaldehyde exposure on lymphocyte MN formation were systematically analysed and published [49] • Angelini et al reported on the results of a systematic review and meta-analysis on studies of lymphocyte MN in populations exposed to petroleum and its derivatives. [50] • A systematic review of published studies showed that the lymphocyte CBMN assay can detect the genotoxic impact of occupation exposure to butadiene [51] • A systematic review of occupational exposure to ethylene oxide and DNA damage biomarkers showed that MN were only observed in a limited number of studies with high exposure and not below the recommended concentration of <1ppm [52] • A systematic review and meta-analysis was published confirming the suitability of the lymphocyte CBMN assay for measuring the in vivo and ex vivo genotoxic effects in humans exposed to styrene [53] • Frequency of MN and other biomarkers of DNA damage in populations exposed to dusts, asbestos and other fibres were assessed and reported in a systematic review [54] • Results of lymphocyte MN assays were systematically analysed in human studies of occupational and environmental exposure to mercury, lead and cadmium [55] • A review of biomonitoring studies focused on lymphocyte MN of people exposed to arsenic, chromium, nickel, vanadium was completed and published [56] • The systematic review of Sram et al [57] investigated results from several studies to determine whether the use of the lymphocyte CBMN assay is suitable to measure DNA damage induced by exposure to polycyclic aromatic hydrocarbons • Studies on lymphocyte MN induction in occupational exposure to coal, dyes, paints, organic solvents in a complex mixture, and others miscellaneous chemicals were analyzed and reported by da Silva [58] • A systematic review of publications on biomonitoring studies using the lymphocyte CBMN assay in people exposed to pesticides in different settings was completed and published [59] • A review was published on the research that still needs to be done to test whether the lymphocyte CBMN assay can be used successfully for measuring the in vivo genotoxic effects of nanomaterials in humans [60] • A summary of the results and other outcomes from the reported epidemiological and meta-analysis review papers in the special issue was also published to provide a synthesis and overview of current knowledge on the use of the lymphocyte CBMN assay to measure DNA damage induced by exposure to genotoxic chemicals [61].	
2017	Completion of an HUMN-coordinated inter-laboratory slide scoring exercise of the buccal micronucleus cytome assay and publication of its results indicating inter-laboratory consistency and variability depends on biomarker scored and laboratory experience.	[62]
2018	A synthesis of systematic reviews was published regarding the validity of the Lymphocyte Cytokinesis-Block Micronucleus Assay as biomarker for human exposure to chemicals with different modes of action.	[63]
2019	In 2019 a book was published on the use of the micronucleus assay in toxicology [64]. This book was edited by two scientists in the HUMN network [and contained many review chapters contributed by HUMN scientists relating to (i) The origins of micronuclei and their association with disease [65-68], (ii) The in vitro and in vivo application of micronucleus assays using cells from humans, rodents and other species [69-85] and (iii) Micronucleus assays and their application to study DNA damage induced by in vivo exposure to genotoxins in humans [86-102].	[64-102]

The specific topics of each chapter are listed below:

- A Short Personal History of Micronuclei [65]
- Mechanisms by which Genotoxins Cause Micronuclei and Other Nuclear Anomalies [66]
- The Fate of Micronuclei and Micronucleated Cells [67]
- Micronuclei and their Association with Infertility, Pregnancy Complications, Developmental Defects, Anaemias, Inflammation, Diabetes, Chronic Kidney Disease, Obesity, Cardiovascular Disease, Neurodegenerative Diseases and Cancer [68]
- The Cytokinesis-Block Micronucleus Cytome Assay in Human Lymphocytes [69]
- Micronucleus Cytome Assay with Buccal Cells [70]
- Experiments with Micronucleus Assays Using Nasal, Urothelial and Cervical Human Cells [71]
- Micronuclei in Bone Marrow Cells of Rodents [72]
- The in vitro Micronucleus Assay with Rodent Hepatocytes [73]
- Micronucleus Assays with Human Lymphocytes for in vitro Genetic Toxicology Testing [74]
- The Micronucleus Assay Using Human-derived Cells [75]
- Micronucleus Experiments with Non-Human Mammalian Cells [76]
- Micronucleus Experiments with Fish Cell Lines [77]
- The Piscine Erythrocyte Micronucleus Cytome Assay [78]
- Micronucleus Experiments with Reptiles [79]
- Micronucleus Assays in Amphibians [80]
- Micronucleus Experiments with Bivalve Molluscs [81]
- Micronucleus Assays with Meiotic Pollen Tetrad Cells of Tradescantia and with Mitotic Root Tip Cells of Allium cepa and Vicia faba [82]
- Automated Analysis of Micronuclei, R. C. Wilkins [83]
- Regulatory Aspects and Guidelines for the Use of Micronucleus Assays in Mammalian and Human Cells [84]
- Micronucleus Assay: Epidemiological and Statistical Issues [85]
- Effects of Tobacco Smoking on Micronucleus Frequency [86]
- Khat, Betel, Coca and Tobacco Chewing: Their Genotoxic Effects Measured Using Micronucleus Assays [87]
- Electromagnetic Fields and Micronuclei [88]
- Use of the Micronucleus Assay in Occupational Studies Involving Exposure to Genotoxic Chemicals - An Overview [89]
- Ionising Radiation Exposure Biodosimetry Using the Lymphocyte Cytokinesis-Block Micronucleus Cytome Assay [90]
- Heavy Metals I – Lead, Mercury and Cadmium and their Impact on DNA Damage Measured by the Micronucleus Assay [91]
- Heavy Metals II - Arsenic, Chromium, Nickel, Vanadium and Micronuclei [92]
- Effect of in vivo Formaldehyde Exposure on DNA Damage Measured by the Micronucleus Assay in Lymphocytes, Buccal Cells and Nasal Cells [93]
- Pesticides Exposure and Effects on Micronucleus Frequency [94]
- Petroleum, its Derivatives and Micronuclei [95]
- Butadiene and its Effects on Micronucleus Frequency [96]
- The Effect of Dust, Asbestos and Other Fibers on DNA Damage Measured Using the Micronucleus Assays [97]
- Use of Micronucleus Assays to Measure DNA Damage Caused by Coal Dust and Ash [98]
- Micronucleus Assay as Cytogenetic Biomarker of Ethylene Oxide Exposure [99]
- Use of Micronucleus Assays to Measure DNA Damage Caused by Cytostatic/Antineoplastic Drugs [100]
- Micronucleus Assay for Assessing Chromosomal Damage of Medical Workers Exposed to Anaesthetic Gases [101]
- Micronucleus Induction by Exposure to Vinyl Chloride [102]

2019	An update on Micronucleus Cytome Assays in Human Lymphocytes and Buccal Cells was published.	[103]
2020	A new study on the effects of smoking on micronuclei and other nuclear anomalies in cervical cells was communicated.	[104]
2020	A report on the HUMN workshop on "Micronuclei and Disease" held in Rennes (France) was published which led to a special issue on this topic a year later (see below)	[105]
2020-2021	A special issue "Micronuclei and Disease" was initiated by the HUMN project to (i) Determine the current level of evidence for association of micronuclei (MN) with risk of specific major diseases including progression, prognosis and genotoxic consequences of therapy and/or its efficacy. (ii) Define plausible mechanisms that may explain association with each disease and evidence that may support it. (iii) Identify knowledge gaps and further work needed to justify translating use of MN and other related nuclear anomalies (e.g. nucleoplasmic bridges, nuclear buds) into clinical practice. The papers in the special issue addressed the following topics: • "Micronuclei and Disease" special issue: Aims, scope, and synthesis of outcomes [106] • Micronuclei as biomarkers of DNA damage, aneuploidy, inducers of chromosomal hypermutation and as sources of pro-inflammatory DNA in humans [107] • Micronuclei, inflammation and auto-immune disease [108] • Micronucleus frequency in chronic kidney disease patients: A review [109] • Chromosomal damage measured by the cytokinesis block micronucleus cytome assay in diabetes and obesity - A systematic review and meta-analysis [110] • Genomic instability in chronic obstructive pulmonary disease and lung cancer: A systematic review and meta-analysis of studies using the micronucleus assay [111] • Micronuclei, reproduction and child health [112] • Micronucleus assay for predicting coronary artery disease: A systematic review and meta-analysis [113] • Micronuclei and upper body cancers (head, neck, breast cancers) a systematic review and meta-analysis [114] • Genomic instability as a main driving factor of unsuccessful ageing: Potential for translating use of MN assays into clinical practice [115] • Impact of infections, preneoplasia and cancer on micronucleus formation in urothelial and cervical cells: A systematic review [116] • Impact of dietary and lifestyle interventions in elderly or people diagnosed with diabetes, metabolic disorders, cardiovascular disease, cancer and micronutrient deficiency on micronuclei frequency – A systematic review and meta-analysis [117] • Association between glycation biomarkers, hyperglycemia, and micronucleus frequency: A meta-analysis [118] • Lymphocyte micronuclei frequencies in skin, haematological, prostate, colorectal and esophageal cancer cases: A systematic review and meta-analysis [119] • Roadmap for translating results from the micronucleus assay into clinical practice: From observational studies to randomized controlled trials [120]	[106-120]
2021	The results of a novel study on the impact of consumption of hot beverages on acute cytotoxic and genotoxic effects in oral mucosa cells measured using the buccal micronucleus cytome assay was reported.	[121]
2021	A review and meta-analysis of published studies indicated that occupational exposure to crystalline silica dust increased MN frequency two-fold.	[122]
2022	A paper on recommendations and quality criteria for micronucleus studies with humans was communicated.	[123]

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