

gH2AX/pH3

Commonly used acronym: gH2AX/pH3

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SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Basic Research, Regulatory use - Routine production
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	Any cell line model or primary cells

DESCRIPTION

Method keywords

Histone-PTMs

genotoxicity

mode of action

H2AX

H3

Scientific area keywords

genotoxicity
aneugen
clastogen
in vitro toxicology
carcinogenicity
Chemical testing

Method description

A battery of *in vitro* genotoxicity assays is currently used to detect agents with DNA damaging and carcinogenic potential. Although the sensitivity of this battery of genotoxicity assays is high, the specificity is low ("false positive hit"), especially with the mammalian cell-based assay, compared with *in vivo* data. Understanding the mode of genotoxic action (MoA: aneugen or clastogen) of a genotoxic chemical is another important requirement. The proposed gH2AX/pH3 method permits the measurement of early biomarkers not only able to predict DNA damage, but also differentiate between numerical and structural chromosome damage. This assay is applicable to almost all cell types including cell of human origin. The assay uses innovative techniques with a higher throughput than usual assays and potentially amenable to automation. The method is less prone to irrelevant positive results as compared to some of the current assays because it is able to also discriminate the misleading cytotoxic chemicals.

Lab equipment

Classical cellular biology equipment.

To analyze the two endpoints (gH2Ax and pH3) different methods are suitable (In Cell Western, FACS, HCA...).

Method status

History of use
Internally validated
Published in peer reviewed journal

Currently submitted for further validation by an external party (e.g. OECD, EURL ECVAM,...)

PROS, CONS & FUTURE POTENTIAL

Advantages

- a) The gH2AX/pH3 method has been reported to be more predictive of genotoxicity (for specificity and sensitivity) than the commonly used assays (MNvit and Ames) and could detect efficiently different types of genotoxic compounds at low concentrations.
- b) This assay is faster than MNvit test because it did not require two cell cycles completion.
- c) This assay is the first one to permit to easily discriminate the genotoxic mode of action very powerfully (clastogens, aneugens and misleading cytotoxic chemicals) in any human cell type. The use of cell lines with different metabolism capacities enabled differentiation between directly from bioactivated genotoxins.
- d) The use of multi well plates allows high-throughput format for both cell treatment and parameter measurement.
- e) The biomarkers and the antibodies used for the activity measurement are not protected by any patent or license and the technologies are relatively inexpensive to perform.

Challenges

The gH2AX/pH3 genotoxic method is highly suitable for the detection of genotoxic properties of substances amenable to solution. Chemicals are routinely dissolved in aqueous solvent such as water or organic solvents such as DMSO. For hydrophobic substances like oil-derived chemicals, DMSO extracts can be tested. As with conventional *in vitro* genotoxicity assays, the gH2AX/pH3 biomarkers do not easily allow the evaluation of gasses. It is hoped that new advances in this area will enable such investigations. The method is able to detect genotoxins that require metabolic activation, providing that metabolically competent cells are used or external metabolic activation system is added (e.g., S9 fraction). Since the gH2AX signal is expected to in part result from DNA replication or transcription blocking lesions induced by genotoxins, cells in a proliferative state may be more appropriate than

cells in a confluence state.

Modifications

The use of high content analysis platform permits to detect in parallel different biomarkers. So it is possible to detect other biomarker of interest than only phosphorylated histone H2AX and H3.

Future & Other applications

The gH2AX/pH3 test method is not aimed to replace or delete an existing method. The gH2AX/pH3 method has the unique possibility to provide mechanistic insights into the mechanism of genotoxicity by combining two relevant endpoints in a single assay. gH2AX/pH3 method can be particularly useful in an Adverse Outcome Pathway (AOP) and in the development of an Integrated Approach to Testing and Assessment (IATA). The mechanistic information that is provided by the gH2AX/pH3 method assay can be applied to translate the Molecular Initiating Events (MIE) and cellular responses that are activated upon chemical exposure to carcinogenicity hazards for humans. Discussion will begin this year at OECD for a official test guideline.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Kopp B, Sanders P, Alassane-Kpembi I, Fessard V, Zalko D, Le Hégarat L, Audebert M. (2020) Synergic toxic effects of food contaminant mixtures in human cells. *Mutagenesis*. 18:geaa019.

Khoury L, Zalko D, Audebert M. (2020) Evaluation of the genotoxic potential of apoptosis inducers with the H2AX assay in human cells. *Mutat Res*. 852:503165.

Kopp B, Le Hégarat L, Audebert M. (2020) Differential toxic effects of food contaminant mixtures in HepaRG cells after single or repeated treatments. *Mutat Res*. 850-851:503161.

Tomasetig F, Tebbby C, Graillot V, Zeman F, Pery A, Cravedi JP, Audebert M. (2020) Comparative genotoxic potential of 27 polycyclic aromatic hydrocarbons in three

human cell lines. *Toxicol Lett.* 326:99-105.

Kopp B, Khoury L, Audebert M. (2019) Validation of the H2AX biomarker for genotoxicity assessment: a review. *Arch Toxicol.* 93(8):2103-2114.

Louisse J, Rijkers D, Stoopen G, Holleboom WJ, Delagrangé M, Molthof E, Mulder PPJ, Hoogenboom RLAP, Audebert M, Peijnenburg AACM. (2019) Determination of genotoxic potencies of pyrrolizidine alkaloids in HepaRG cells using the H2AX assay. *Food Chem Toxicol.* 131:110532.

Bryce, S.M., Bernacki, D.T., Bemis, J.C., Spellman, R.A., Engel, M.E., Schuler, M, Lorge, E., Heikkinen, P.T., Hemmann, U., Thybaud, V., Wilde, S., Queisser, N., Sutter, A., Zeller, A., Guérard, M., Kirkland, D., Dertinger, S.D., 2017. Interlaboratory evaluation of a multiplexed high information content in vitro genotoxicity assay. *Environ Mol Mutagen* 58, 141-161.

Chevereau, M., Glatt, H., Zalko, D., Cravedi, JP., Audebert, M., 2017. Role of human sulfotransferase 1A1 and N-acetyltransferase 2 in the metabolic activation of 16 heterocyclic amines and related heterocyclics to genotoxicants in recombinant V79 cells. *Arch Toxicol* , in press.

Khoury, L., Zalko, D., Audebert, M., 2016. Complementarity of phosphorylated histones H2AX and H3 quantification in different cell lines for genotoxicity screening. *Arch Toxicol* 90, 1983-1995.

Khoury, L., Zalko, D., Audebert, M., 2016. Evaluation of four human cell lines with distinct biotransformation properties for genotoxic screening. *Mutagenesis* 31, 83-96.

Quesnot, N., Rondel, K., Audebert, M., Martinais, S., Glaise, D., Morel, F., Loyer, P., Robin, M.A., 2016. Evaluation of genotoxicity using automated detection of H2AX in metabolically competent HepaRG cells. *Mutagenesis* 31, 43-50.

Bernacki, D.T., Bryce, S.M., Bemis, J.C., Kirkland, D., Dertinger, S.D., 2016. H2AX and

p53 responses in TK6 cells discriminate promutagens and nongenotoxicants in the presence of rat liver S9. *Environ Mol Mutagen* 57, 546-58.

Bryce, S.M., Bernacki, D.T., Bemis, J.C., Dertinger, S.D., 2016. Genotoxic mode of action predictions from a multiplexed flow cytometric assay and a machine learning approach. *Environ Mol Mutagen* 57, 171-189.

Bryce, S.M., Bemis, J.C., Mereness, J.A., Spellman, R.A., Moss, J., Dickinson, D., Schuler, M.J., Dertinger, S.D., 2014. Interpreting in vitro micronucleus positive results: simple biomarker matrix discriminates clastogens, aneugens, and misleading positive agents. *Environ Mol Mutagen* 55, 542-555.

Khoury, L., Zalko, D., Audebert, M., 2013. Validation of high-throughput genotoxicity assay screening using H2AX in-cell western assay on HepG2 cells. *Environmental and Molecular Mutagenesis* 54, 737-746.

Audebert, M., Zeman, F., Beaudoin, R., Pery, A., Cravedi, J.P., 2012. Comparative potency approach based on H2AX assay for estimating the genotoxicity of polycyclic aromatic hydrocarbons. *Toxicol Appl Pharmacol* 260, 58-64.

Associated documents

[Kopp_ArchTox2019review.pdf](#)

Other remarks

Since 2008 and the first experiment conducted in our lab, for each cell line, in each experiment, the same benchmark positive control is included. The assay has been done by ten different experimenters with consistently high reproducibility (superior to 95%), independent of the cell line used. The gH2AX/pH3 method was transferred successfully to five academic laboratories (RIKILT, Netherland; Laboratory of Toxicological Control of Pesticides, Greece; Institut für Toxikologie, Deutschland; IPBS-CNRS UMR5089, France; INSERM U1220-INRA U1416, France). An inter-laboratory trial of the gH2AX/pH3 method was performed with seven different private companies (Litron, Pfizer, Servier, Orion, Sanofi-Aventis, Bayer and Roche Pharma) using 84 chemicals. The validation has been performed largely according

to OECD Guidance document 34 on the validation and international acceptance of new test methods for hazard assessment. An overall concordance between companies of 92% was achieved with a sensitivity of 92% and a specificity of 96%. (see first paper in the References section)

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