Alkaline In Vitro Comet Assay in C3a cells

Commonly used acronym: *in vitro comet*

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Contact person

Roel Anthonissen

Organisation

Name of the organisation Sciensano
Department Chemical and physical health risks
Country Belgium

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	C3a Cells (Hepatocellular Carcinoma Cells, the C3A cell line is a clonal derivative of Hep G2 cells)

DESCRIPTION

Method keywords

DNA damage
DNA strand breaks
single cell gel electrophoresis assay
comet
automated scoring

Scientific area keywords

in vitro toxicology
genotoxicity
hazard assessment

Method description

The in vitro Alkaline Comet Assay is a microgel electrophoresis technique which allows to measure DNA damage (single and double strand breaks, alkali labile sites, incomplete excision repair sites and cross links) cell by cell. Cells are mixed with 0.8% Low Melting Point Agarose which is spread as a gel onto a microscope slide. The cells are lysed with high salt concentrations and detergents. The remaining nuclear DNA is then denaturated

in alkali buffer pH>13 and electrophoresed in the same buffer. The negatively charged DNA fragments migrate out of the nucleus, towards the positive pole whereas undamaged supercoiled DNA does not migrate. After electrophoresis and neutralization of the slides, cells are dried and stained with GelRed, a fluorochrome intercalating agent. A fluorescent microscope equipped with an image analysis system is used to capture and quantify DNA damage in the single cells. DNA damage is expressed as percentage of DNA in the tail.

Lab equipment

- Standard equipment for working with cell cultures;
- Electrophoresis Chamber with Power Supply Circulating Pump;
- Fluorescence Microscope;
- Software for automated imaging.

Method status

History of use
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Identify DNA damage at the single cell level;
- Sensitivity for detecting low levels of DNA damage;
- Only small numbers of cells per sample needed;
- Fast, cheap & easy;
- Applicable on many cell types.

Challenges

- Most of the damage is repaired (no fixed mutations are detected, regulatory genotoxicity testing in vitro usually relies on mutagenicity test not indicator tests) but this gives an opportunity to study DNA repair;
- Quality of protocol and experimental performance is of crucial importance;
- Suitable statistical analysis;
- Incomplete metabolic capacities C3a cell line (Problems with indirect mutagens).

Modifications

- Use of a cooling system for the electrophoresis device to minimize slide/slide differences;
- Improvement of the imaging system (upgrade software metasystems);
- Use of other cell lines (e.g. TK6 cells).

Future & Other applications

- High Throughput analysis (48 well slides Trevigen);
- Use of lesion specific enzymes to make comet more sensitive (eg FPG) and more specific (eg epigenitic studies).

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Collins AR, Azqueta Oscoz A, Brunborg G, Gaivão I, Giovannelli L, Kruszewski M, Smith CC, Stetina R (2008) The comet assay: topical issues. Mutagenesis 23:143–151.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35:206–221.
Azqueta A. and Collins A. R . (2013) The essential comet assay: a comprehensive guide to measuring DNA damage and repair. Arch. Toxicol ., 87, 949–968.
Investigation into the genotoxicity of water extracts from Hypoxis species and a commercially available Hypoxis preparation., Verschaeve, Luc, Mertens Birgit, Ndhlala A R., Anthonissen R, Gorissen B, and Van Staden J , Phytother Res, 2013 Mar, Volume 27, Issue 3, p.350-6, (2013).

Associated documents

The essential comet assay a comprehensive guide to measuring DNA damage and repair.pdf
Single Cell GelComet Assay Guidelines for In Vitro and In Vivo Genetic Toxicology Testing.PDF
the comet assa topical issues Mutagenesis-2008-Collins-143-51.pdf
Investigation into the genotoxicity of water extracts from Hypoxis species and a commercially available Hypoxis preparation.pdf

Other remarks

In addition to genotoxicity testing we also apply the in vitro comet assay to examine the antigenotoxic properties of test substances (mostly plant extracts). A test solution is considered antigenotoxic when the genetic damage caused by the combined treatments (extracts and known mutagen) is substantially lower compared to the damage induced by the mutagen alone.

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