

VITOTOX assay

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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

DESCRIPTION

Method keywords

genotoxicity
DNA damage
SOS response
luminescence
genetically modified bacterial strains
screening

Scientific area keywords

in vitro toxicology
genotoxicity

cytotoxicity

hazard assessment

Method description

The VITOTOX test is a bacterial genotoxicity test. The test is based on bacteria that contain the lux operon of *Vibrio fischeri* under transcriptional control of the mutated recN promoter, which is controlled by the bacterial SOS-system (TA 104-recN2-4 strain or Genox strain). After incubation of the cells with a genotoxic product, the recN promoter will become derepressed, resulting in expression of the lux operon. This expression results in light production in function of the genotoxicity. Some products act directly on the light production (e.a aldehydes) or enhance the metabolism of the bacteria, creating false-positive results. Therefore a bacterial strain with a constitutively expressed lux operon (pr1-lux fusion = continuous light production) is also used as an internal control (pr1 or Cyttox strain). If the light production increases in this Cyttox strain, the test compound affects the lux gene in a different way than by damaging the DNA. Furthermore, a decrease in light production would indicate a cytotoxic response. The bacteria in both strains are treated with the test substance in the presence and absence of a metabolizing induced rat liver S9 fraction (for detection of metabolites). Light measurements are performed in a luminometer at constant temperature every 5 minutes in each well of a black 96-well plate during a 4-hour period after addition of the test samples to the bacteria. The signal to noise ratio (S/N) or, specifically, the light production of exposed bacteria divided by the light production of non-exposed bacteria is calculated for each measurement. S/N is calculated for both strains separately and the ratio between the maximum S/N values of the genox over the cytox strain. A substance is considered genotoxic when: - $\max S/N (\text{genox})/\max S/N (\text{cytox}) > 1.5$ - $\max S/N$ in genox shows a good dose-effect relationship - $\max S/N (\text{genox})/\max S/N (\text{cytox})$ shows a good dose-effect relationship. A substance is considered cytotoxic when S/N in the cytox strain decreases and becomes considerably lower than 0.8. If both strains are (strongly) induced there is no evidence for genotoxicity.

Lab equipment

- Standard equipment for working with cell cultures;
- Luminometer;

- Shaking Incubator.

Method status

History of use

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- VITOTOX correlates well with the Ames assay and shows high specificity and sensitivity;
- The test is useful for rapid screening of large numbers of chemicals when only a small quantity of a chemical is available;
- Detection of and distinction between direct acting mutagens and pro-mutagens that need metabolization.

Challenges

- Less appropriate for testing the genotoxicity of complex mixtures;
- Exogenous metabolic system is of animal origin (induced rat or hamster S9 liver fraction) and might be different from human metabolism;
- Not able to detect aneuploidy-inducing agents;
- False positive results with compounds that interfere with light production (eg aldehydes) or enhance the metabolism of the bacteria.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

D. Lelie van der, L. Regniers, B. Borremans, A. Provoost, L. Verschaeve, The VITOTOX test, an SOS bioluminescence *Salmonella typhimurium* test to measure genotoxicity kinetics, *Mutat. Res.* 389 (1997) 279–290.

L. Verschaeve, J. Van Gompel, L. Thilemans, L. Regniers, P. Vanparys, L.D. van der, VITOTOX bacterial genotoxicity and toxicity test for the rapid screening of chemicals, *Environ. Mol. Mutagen.* 33 (1999) 240–248.

Genotoxicity and Antigenotoxicity of selected South African indigenous plants EEE R. Makhuele, R.G. Matshoga, R. Anthonissen, L. Pieters, L. Verschaeve *South African*

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Muto S, Baba H, Uno Y. Evaluation of the VITOTOX™ test as a high-throughput genotoxicity assay. Environment Mutagenesis Research. 2003;25:69–75.

Associated documents

[The VITOTOX test, an SOS bioluminescence Salmonella yphimurium test measure genotoxicity kinetics.pdf](#)

[Genotoxicity and Antigenotoxicity of selected South African indigenous plants.pdf](#)

[VITOTOX Bacterial Genotoxicity and Toxicity Test for the Rapid Screening of Chemicals.pdf](#)

[Evaluation of the Vitotox™ test as a high-throughput genotoxicity assay.pdf](#)

Other remarks

In addition to genotoxicity testing, we also use the VITOTOX test 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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