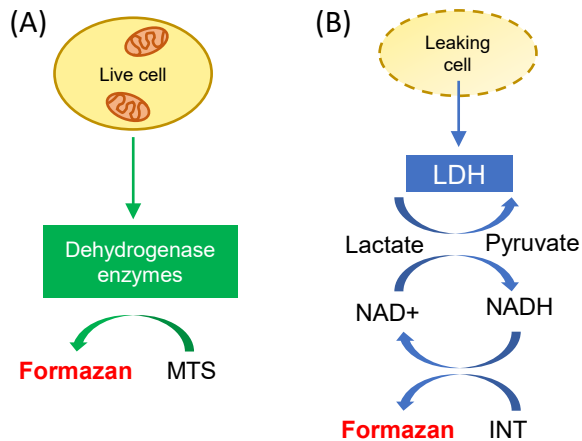


Introduction

Cytotoxicity testing is an integral part of drug, medical device, and probiotic product development. Spectrophotometer-based colorimetric assays are commonly employed because of their ease of use and rapid turn-around. While the chemical mechanism of different colorimetric assay can vary, in general, the reagent undergoes a chemical (and thus, colorimetric) change in the presence of cellular enzyme(s). In this poster we will highlight the MTS assay¹, adapted from ISO 10993-5, and the LDH assay², reference in the 2018 EFSA FEEDAP guideline. Both assays are based on the conversion of a tetrazolium salt (MTS or INT, respectively) to a red formazan product. However, MTS assay measures viable cells via cellular metabolic activity, while LDH assay measures cell death via the release of intracellular LDH³.

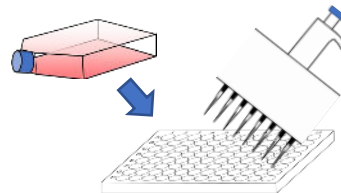
Reaction Mechanism



(A) In the MTS assay, dehydrogenase enzymes in live, metabolically active cells convert MTS to formazan. (B) In contrast, release of cytosolic LDH from dead and dying cells are used to measure cell death in LDH assay, where LDH facilitates the conversion of INT to formazan.

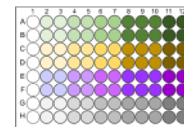
ISO 10993-5 MTS Assay Workflow

Adapted from ISO 10993-5, the general MTS assay workflow depicted below is used to assess cytotoxicity of leachable substances from medical devices. In addition to leachables from medical devices, similar workflow have been used to test a range of different compounds and products.



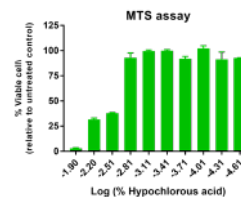
Step 1) Seed L-929 cells in 96-well microplate and grow until ~50% confluency (approx. in 24 hours).

Step 2) Remove media and dispense test articles at different concentrations to cells; include untreated control. Incubate for 24 hours.



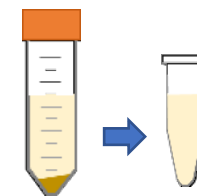
Step 3) Perform visual check to confirm viability on an inverted microscope. Once confirmed, remove media from cells and add MTS reagent. Incubate and read absorbance at OD_{490nm}.

Step 4) Absorbance data is used to calculate percentage of viable cell relative to untreated control (example to the right).

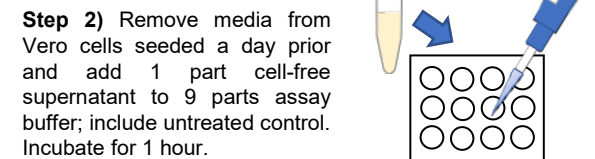


EFSA FEEDAP LDH Assay Workflow

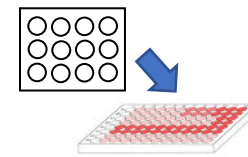
LDH assay is listed as one of the cytotoxicity assay method in EFSA FEEDAP's 2018 guidelines on characterization of microorganisms for use in animal feed. While the workflow below is designed to assess possible cytotoxicity from bacterial extracellular toxins, it can also be adapted to assay other test materials.



Step 1) Grow test organism in media, centrifuge and save the supernatant for LDH testing. As endogenous LDH can also be present in the media, always include a media control in the assay.

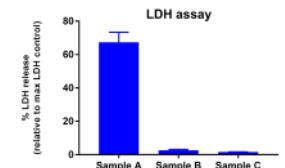


Step 2) Remove media from Vero cells seeded a day prior and add 1 part cell-free supernatant to 9 parts assay buffer; include untreated control. Incubate for 1 hour.



Step 3) Visually assess cell viability. Lyse control cells to obtain max lysis control. Move solution to new microplate and add LDH reagent. Read absorbance at OD_{490nm}.

Step 4) Calculate percentage of LDH release relative to max LDH release control as a readout of cell death (example to the right)



Citations

- ISO 10993-5, 2009, Biological evaluation of medical devices, part 5: test for in-vitro cytotoxicity.
- EFSA FEEDAP, 2018, Guidance on the characterisation of microorganisms used as feed additives or as production organisms.
- In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages, <http://dx.doi.org/10.5772/intechopen.7192>