

## HepaRG spheroid models for predicting liver toxicity

Created on: 14-04-2025 - Last modified on: 23-04-2025

### Organisation

**Name of the organisation** Vrije Universiteit Brussel (VUB)

**Department** Department of Pharmaceutical and Pharmacological Sciences

**Country** Belgium

**Geographical Area** Flemish Region

### SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Translational - Applied Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Species from which cells/tissues/organs are derived</b>	Human
<b>Type of cells/tissues/organs</b>	Immortalized hepatic cell line

### DESCRIPTION

#### Method keywords

HepaRG  
spheroids  
liver cells  
hepatic cell line  
3D in vitro model  
liver model

#### Scientific area keywords

Cholestatic drug-induced liver injury  
cDILI  
Drug-induced cholestasis index  
DICI  
predictive toxicology

#### Method description

Cholestatic drug-induced liver injury (cDILI) is a frequent reason for drug failure and withdrawal during premarketing and postmarketing stages of drug development. Strategies for reliable detection of cDILI in early drug development are therefore urgently needed. The drug-induced cholestasis index (DICI) concept was previously introduced as

a tool for assessing the cholestatic potential of drug candidates. DILI is calculated as the ratio between the viability values obtained in drug-treated liver cells in the presence and absence of bile acids. The present in vitro study was set up to investigate the applicability of DILI in a novel high-throughput and large sample setting. Furthermore, the improvement of the predictivity of the DILI by introduction of advanced modeling was explored. Fifty-eight well-documented drugs were selected and categorized as drugs inducing cDILI, non-cholestatic DILI (ncDILI), and not inducing DILI (non-DILI). Differentiated HepaRG cells were handled according to the manufacturer's instructions. Briefly, thawed cells were centrifuged and resuspended in MHTAP medium. Cells were seeded in ultra-low attachment plates. Plates were maintained for 1 day on a tilting stand to facilitate the aggregation of the cells and improve uniform spheroid formation at 37 °C under a humidified atmosphere containing 5% CO<sub>2</sub>. Cell culture medium was replaced with MHPIT medium and cultured for 7 additional days with cell culture medium renewal every 2–3 days until spheroid formation and maturation. Morphological features and viability of the fully formed spheroids were assessed by phase-contrast imaging at days 0 following spheroid generation (i.e., 8 days after seeding). The average diameter of the spheroids at day 0 after spheroid generation was  $189.1 \pm 9.4 \mu\text{m}$ . HepaRG spheroids showed BSEP protein expression as an indicator of their suitability to investigate cholestatic liability.

### Lab equipment

Centrifuge, Incubator, Confocal imaging system.

### Method status

Published in peer reviewed journal

## PROS, CONS & FUTURE POTENTIAL

### Advantages

- The 3D culturing has shown to increase the similarities in metabolic activities and functional characteristics to the human liver,
- HepaRG cells in 3D spheroid culture show increased sensitivity to DILI drugs compared to 2D cultures, showing high potential to detect cholestatic drugs, based on cytotoxicity and DILI,
- HepaRG spheroids have showed to improve hepatic functionality and metabolism compared to 2D, exhibiting liver specific functions and increasing the sensitivity to hepatotoxic compounds, ultimately allowing for accurate toxicity testing,
- Possibility for chronic exposure up to 14 days compared to the commonly assessed 24–72 hours in monolayer models addressing drug-induced cholestasis index (DILI).

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### Links

[Optimization of the drug-induced cholestasis index based on advanced modeling f...](#)  
[Prof. Mathieu Vinken - Team](#)  
[IVTD - VUB](#)

Coordinated by



Financed by



Vlaanderen  
verbeelding werkt

