

The C-DILI™ Assay is a novel *in vitro* method to evaluate a compound's potential for cholestatic drug-induced liver injury (DILI).

The C-DILI Assay has demonstrated high *in vitro* – *in vivo* correlation with compounds known to have a risk for cholestatic hepatotoxicity. It is an effective tool for assessing the risk of cholestatic DILI and providing data useful in lead selection and managing toxicity risk.

At high concentrations, bile acids can cause liver toxicity by signaling apoptotic pathways as well as disrupting membranes. The liver tightly controls bile acid concentrations through multiple regulatory mechanisms including basolateral efflux, biliary efflux, synthesis, and metabolism. However if control mechanisms are disrupted, bile acid concentrations can reach a “tipping point” resulting in hepatotoxicity. The C-DILI Assay combines all of these human-relevant processes in one simple threshold readout, providing a unique clinically-predictive assessment of cholestasis risk.

## Our Process

TRANSPORTER CERTIFIED™ hepatocytes are cultured in sandwich-culture to re-establish physiologically-relevant uptake, metabolism, regulation and efflux function. The hepatocytes form a matrix with bile pockets and demonstrate transporter function, including BSEP, OSTs, and MRP3/4.

After the culture has been established, BioIVT's proprietary QUALGRO™ Sensitization Media is added to the wells, along with the test compound, and incubated for 24 hours. Cholestatic hepatotoxicity is evaluated by measuring lactate dehydrogenase (LDH) concentration and ATP content versus controls in a standard plate-reader assay.

The response of the test article is compared to drugs with known clinical cholestatic effects. Comparison with negative controls and positive controls allow compounds to be bucketed for their clinical cholestatic hepatotoxicity potential. In addition, an incubation without the QUALGRO Sensitization Media can be performed in parallel to assess hepatotoxicity directly from the compound.

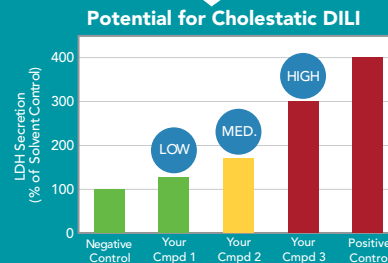
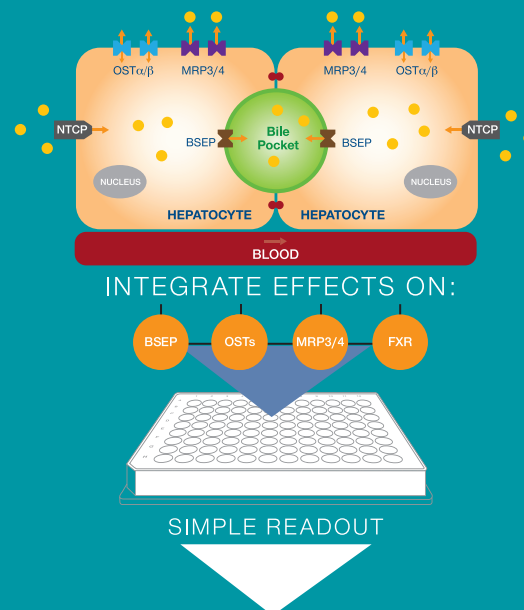
## Study Report

The C-DILI Assay report is an easy-to-read assessment of LDH secretion and ATP content as a percent of solvent control, and compared to negative and positive control standards. Test articles are coded as green, orange, or red, for low, medium or high risk of cholestatic DILI potential.

## A Breakthrough Assay

*In vivo* models are inadequate because rats and dogs have different bile acid constituents and metabolism than humans, with different transporter regulation.

The C-DILI Assay is specially constructed to use a simple LDH and ATP readout that is specific to compounds that inhibit bile acid transport and antagonize FXR and ATP readout. The readout provides a threshold value, and is analogous to clinical tests for liver toxicity.



## Applications

The C-DILI™ Assay integrates effects on BSEP, OST#/#, MRP3/4, and FXR to delineate hepatotoxicity resulting from a build-up of intracellular bile acids and has been shown to be effective in predicting compounds that have clinical hepatocellular cholestatic toxicity.

|                         | Discovery  | Pre-clinical   | Clinical   |
|-------------------------|--|--|--|
| Clinical Concentration  | Unknown  | Estimated  | Known  |
| Screening Concentration | High concentrations (50 – 100 µM). Follow-up hits with a dose ranging study. | Clinical $C_{max}$ or $C_{ss}$ and up to 20X to 50X to account for higher portal vein concentrations | 20X clinical $C_{max}$ or $C_{ss}$ of test compound and anticipated concentration range for co-administered compound |
| Inform Decision-Making  | Lead selection and optimization  | Determine potential for cholestatic hepatotoxicity risk  | Evaluate potential for drug interaction cholestatic hepatotoxicity risk  |

## Accelerate Decision-Making

Either as a stand-alone program or implemented with other studies the C-DILI Assay is designed to complement your R&D process. From early stage screening through optimization and characterization the C-DILI Assay informs your SAR, lead selection, and toxicity assessment programs. An added advantage is that the same system, and even the same cells, can be used for more in depth studies later in your characterization work. The data are all connected and consistent so there is no need to translate data from different model systems.

## Quality

The C-DILI Assay is conducted in BioIVT's research laboratory in Research Triangle Park, NC and is overseen by research scientists with extensive experience in *in vitro* models.

## QUALGRO Sensitization Medium

QUALGRO™ Sensitization Medium is a cell culture medium which creates conditions that cause hepatocytes to respond to the potential cholestatic hepatotoxic effects of a compound.

### For Inquiries:

North America & Asia Pacific  
 T +1 516 483 1196 F +1 516 483 4683  
 E customerservice@bioivt.com

Europe, Middle East & Africa  
 T +44 (0) 1444 250010 F +44 (0) 1444 250066  
 E cseurope@bioivt.com