

General Protocol using TruVivo® for Intrinsic Hepatic Clearance Evaluation

Purpose

The purpose of this bulletin is to provide instructions and recommendations for the proper handling and use of the TruVivo culture system for *in vitro* intrinsic hepatic clearance.

Note: Portions of this protocol must be carried out in advance of dosing.

Precautions

Observe universal precautions when handling human-derived tissues and cells as they are potentially biohazardous and wear appropriate PPE. Refer to the guidelines set forth in Occupational Safety and Health Standards for handling blood, tissues, body fluids, or other potentially infectious materials. Follow institutional guidelines for the collection and disposal of all solid and liquid waste that has been in contact with these products.

Methods

Note: The TruVivo 2D+ Hepatic System, 24-well format* should be prepared following specified instructions for use. The following Intrinsic Hepatic Clearance assay is recommended to be performed between days 5 and 14 of culture.

1. Prepare all test article and reference compound stock solutions using appropriate solvent at 1000x the desired working solution (e.g. 1 μ M). See Table 1 for representative compounds and clearance values obtained when using the TruVivo culture system and protocol.

NOTE: Keep stocks sterile and use appropriate solvents to maximize substrate solubility while minimizing inhibition of CYP enzymes (e.g. DMSO, ACN, methanol).

2. Prepare collection tubes or deep-well plates for analytics (e.g. LC-MS/MS).

Note: All remaining steps should be performed using sterile technique.

3. Dilute the stock test article and reference compound solutions 1:1000 in warmed (37°C)**, TruVivo Culture Medium (TCCM).

NOTE: Keep medium away from overhead or UV light and limit time in water bath to less than 60 min.

4. Remove the matured (day 5-7) TruVivo (and feeder cell only) cultures from the CO₂ incubator.
 5. Aspirate culture medium from row 1 and immediately replace with 500 μ L of the desired working test article or reference compound solutions.
 6. Sample 25 μ L of the working solution (T0) into the prepared collection tubes or corresponding deep-well plate for analytics and store (recommended -80°C).
 7. Repeat dosing and sampling for the remaining rows, with 1-minute intervals between dosing of each row to ensure consistent collection time intervals.
 8. Return the dosed TruVivo culture to the CO₂ incubator.
 9. At designated collection times, sample 25 μ L of TruVivo culture medium from each well and place in the prepared sample tubes for analytics and store (recommended -80°C).
- NOTE:** Sampling should be performed by row, with 1 minute spacing between each row to ensure consistently timed

collections.

(Note Cont): The sampling times should be at 24-h intervals for experiments that extend over 3-5 days (48-72-h intervals for experiments extending to 5+ days) and sufficiently spaced to capture the clearance kinetics of the test article (typically, >10% over a 3–7-day period depending on the metabolic stability of the compound).

10. Using the appropriate analytical methods, determine the concentration of the test article or reference compound in medium at each collection time point and calculate the natural logarithm of the drug fraction remaining (% parent remaining). i.e. $\text{LN}(\% \text{ remaining})_{t_{\text{eq}}} = \text{LN}(\text{concentration}_{t_{\text{eq}}}/\text{concentration}_{\text{working}})$
11. Plot LN (% remaining) versus time (min) and calculate the slope of linear regression.
12. Calculate the half-life and CL_{int} values using established methods (Obach et al., 1999; Sohlenius-Sternbeck et al., 2010).

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Table 1: Representative Intrinsic Hepatic Clearance Reference Compounds and Predicted CL_{int} and $t_{1/2}$ using the TruVivo culture system.

Category	Compound	Final Concentration (μM)	Predicted CL_{int} ($\mu\text{L} * \text{min}^{-1} * 10^6 \text{ cells}^{-1}$)	Predicted $t_{1/2}$ (hours)
High Clearance	Naloxone	1	11.91 ± 0.25	1.62 ± 0.03
Medium Clearance	Diclofenac	1	0.83 ± 0.06	23.51 ± 1.71
Low Clearance	Disopyramide	1	0.15 ± 0.02	130.39 ± 18.50

Materials

1. Analytical balance
2. 37°C Water bath (operating range 35-38°C)
3. Humidified CO2 incubator set for 36.5-37.5°C and 5% CO2
4. Vortex shaker (for tubes)
5. Centrifuge capable of up to 400 x g
6. Timer
7. Multiple pipettes (i.e. 10, 200, 1000 μL)
8. Multiple pipette tips
9. Freezer (-80°C; optional)
10. Multichannel pipette with tips (optional)
11. Serological pipettor (i.e. Pipet-Aid)
12. 1, 2, 5, 10, 25 mL Sterile serological pipettes
13. Sterile plastic or glass aspiration tips and vacuum aspiration system
14. Sterile 50 or 100 mL reagent reservoir
15. 24-well collagen coated plates (e.g. Gibco Catalog # A11428-03)
16. Sterile polypropylene sample tubes (i.e. 1.5 mL Eppendorfs, 15 mL Falcons) or 96-well plate, 2 mL deep well, square (Axygen™, Catalog #P2MLSQC) with sealing mats for 2 mL 96-well plates with square wells (AxyMats™; Axxygen™, Catalog #AM2MLSQ)
17. Disposable plastic or sterile bottles for media preparation (100-250 mL)
18. PES (polyethersulfone) filter units, 0.2 μm (Thermo Scientific, Catalog # 564-0020 [50 mL], 565-0020 [150 mL], 568-0020 [250 mL], or 569-0020 [500 mL]; or MilliporeSigma, Catalog # SCGP00525 [50mL])
19. TruVivo 24-Well Kit + Media
20. Cellometer Spectrum cell counter (optional) (e.g. Nexcelom; SN #Spectrum 101-0030, #Spectrum 101-0031, or #Spectrum 101-0055)
21. Inverted microscope with brightfield optics (or phase contrast – optional)

References

Obach et al, Drug Metab Dispos. 1999;27(11):1350-9; Sohlenius-Sternbeck et al, Xenobiotica. 2010 Sep;40(9):637-49.

* This protocol is designed for 24-well format. If you need instructions specific for 96-well format, please contact your LifeNet Health LifeSciences Sales representative or email lifesciences@lifenethealth.org for technical support.

**Complete media is the base medium with recommended supplements (MED-TCCM plus MED-TCSA and MED-TCSC). Specific protein concentrations and/or recommendations available upon request.