LifeNet Health® LifeSciences

TruVivo® Application Brief:

Advanced Hepatic Modeling for More Reliable Low-Clearance Evaluation

The Research Challenge

Drug developers rely on a variety of hepatic models to evaluate metabolic stability and predict human clearance, yet standard short-term *in vitro* systems often fall short for very stable or low-turnover compounds.[1]

Non-CYP pathways—particularly aldehyde oxidase (AO)—are even more challenging, as many advanced hepatocyte models tend to show low or unstable AO activity.[2] This results in poor IVIVE, underpredicted human clearance, and in some notable cases, unanticipated AO-driven metabolites or clinical failures.[2]

Researchers need a hepatic model capable of:

- Supporting a high cell-to-medium per well ratio over prolonged exposures to compounds
- Maintaining stable CYP and non-CYP activity over time in culture, including AO
- Delivering accurate clearance metabolism predictions across diverse chemotypes

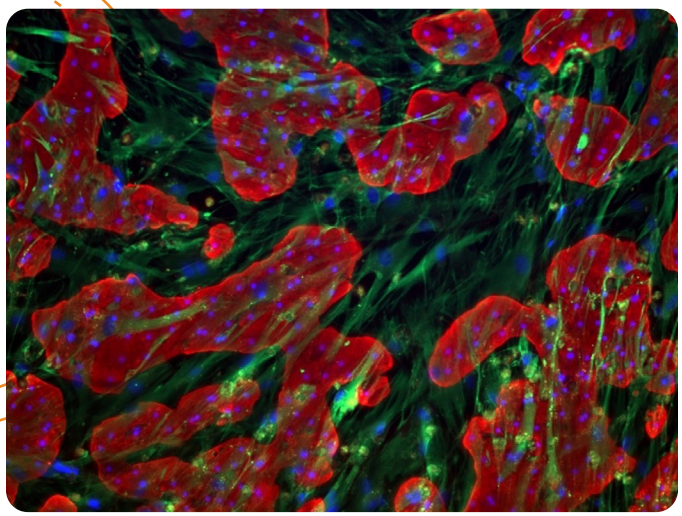

Figure 1. Representative confocal laser microscopy image of TruVivo (blue: nuclei [DAP], green: stromal cells [vimentin], and red: hepatocytes [cytokeratin 18] revealing self-assembled hepatocyte colonies that mimic the microarchitecture of the human liver.[3]

The TruVivo® Solution

TruVivo is an advanced 2D+ human hepatic system designed to deliver sustained metabolic function over extended incubation periods. Its combination of hepatocytes with endothelial and stromal cells maintains enzyme activity—including AO—at levels suitable for reliable clearance assessment and metabolism profiling.

TruVivo addresses the key limitations of current systems:

- **Sustained metabolic activity:** CYP, UGT and AO levels stabilize by Day 3 and remain elevated through Day 14, enabling quantifiable CYP and non-CYP metabolism.[2]
- **Extended assay windows:** Supports 7-day and longer incubations, overcoming the limitations and "insufficient turnover" seen in suspension models.[1,2]
- **Improved measurement of low-clearance compounds:** Only 4% of substrates tested show insufficient turnover in TruVivo compared to 40% in suspension hepatocytes.[1]
- **Consistent performance:** Reduced donor variability and improved reproducibility relative to suspension or monoculture systems.[2]

The result: A stable, human-relevant platform that improves clearance prediction of low-turnover compounds and more reliably captures both CYP and non-CYP metabolism, including AO-mediated reactions.

Key Findings and Validation

Key Finding	Supporting Evidence
TruVivo enables reliable turnover and clearance assessment for low-clearance compounds	TruVivo demonstrated sufficient turnover for 96% of compounds tested, a substantial improvement over suspension hepatocytes (60%).[1] TruVivo also supported multi-day incubations and quantifiable depletion for metabolically stable substrates that could not be measured in traditional models.[2]
Improved IVIVE accuracy across diverse chemotypes compared with traditional models	TruVivo predicted *in vivo* blood clearance within 3-fold for 60% of compounds in a 50-compound panel.[1] TruVivo achieved 4/5 substrates within 3-fold of observed CL_h.[2]
Higher and more stable metabolic enzyme activity compared to traditional plated sandwich cultures (SC)	TruVivo maintained stable CYP and AO activity for ≥14 days, whereas SC showed rapid decline within 24-48 hours.[2] TruVivo supported higher CYP1A2, 2B6, and 3A4 activity compared to SC.[3]
Reduced donor-to-donor variability and improved reproducibility	TruVivo showed minimal donor variance (<5% of compounds varied >3-fold), outperforming preload and suspension assays.[1]
Sustained AO activity for ≥14 days enabling reliable evaluations of AO metabolism	AO activity remained elevated between Days 3 and 14 in TruVivo, outperforming suspension and SC models.[2]
Accurate clearance prediction for AO substrates	TruVivo predicted carbazeran, zoniporide, zaleplon, and O6-BG clearance values within ~2-fold (WSM/PTM) and within 3-fold for 4/5 tested AO substrates.[2]
Reliable $f_{m,AO}$ estimation and AO-metabolite profiling	$f_{m,AO}$ predictions for zaleplon and zoniporide were within 25% of *in vivo* values. TruVivo supported long-term quantification of AO-specific metabolites (e.g., 4-oxo-carbazeran, 8-oxo-O6BG) over 14 days.[2]

	$CL_{int,u}$		CL_b	
	SH	**TruVivo**	**SH**	**TruVivo**
Insufficient Turnover	41%	4%	40%	4%
<2-fold	16% (8/29)	34% (17/47)	20% (10/30)	38% (19/48)
<3-fold	20% (10/29)	49% (24/47)	32% (16/30)	60% (30/48)
3-10-fold	22% (11/29)	39% (19/47)	16% (8/30)	30% (15/48)
>10-fold	16% (8/29)	8% (4/47)	12% (6/30)	6% (3/48)

Table 1 (Above). TruVivo Improves Turnover Success Rates Compared to Suspension Hepatocytes. Comparison of turnover outcomes in suspension hepatocytes (SH) and TruVivo for a 50-compound metabolic stability panel, adapted from Kukla *et al*. TruVivo demonstrated sufficient turnover for 96% of compounds, whereas SH achieved sufficient turnover for only 60%. These results reflect the extended metabolic stability and higher assay success rate of TruVivo, supporting more reliable clearance estimation for low-clearance and metabolically stable compounds. Table reproduced from portions of Table 1 of Kukla *et al*. with permission under an open access license.[1]

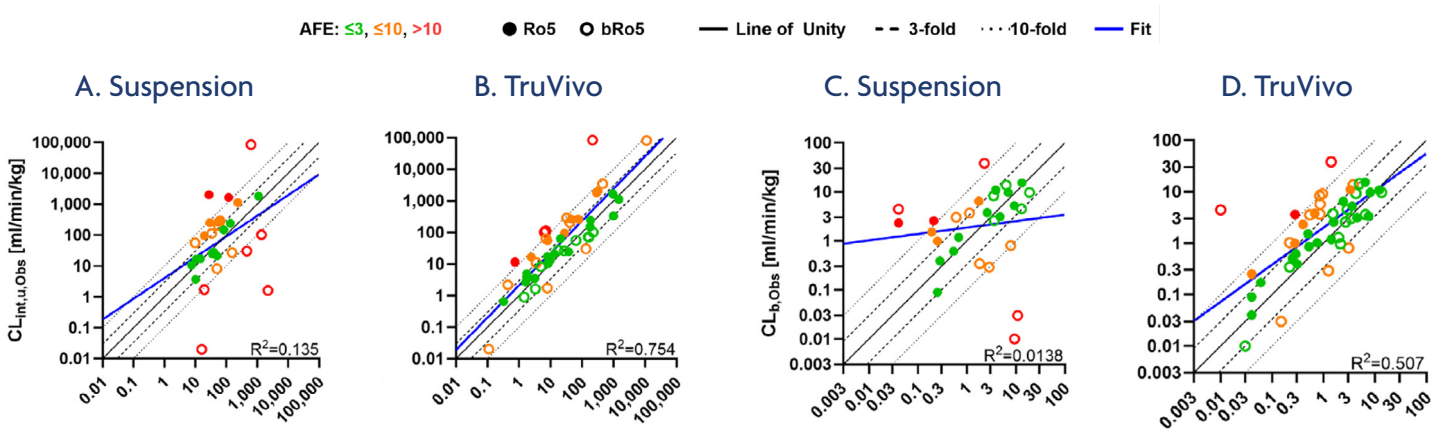

Figure 2 (Above). Comparison of *in vitro-in vivo* correlation of hepatic clearance for 50 compounds using TruVivo and conventional suspension hepatocytes adapted from Kukla *et al*. Predicted intrinsic clearance ($CL_{int,u}$, panels A and B) and blood clearance (CL_b, panels C and D) values are plotted against observed *in vivo* values. Symbols indicate compounds predicted within 3-fold (green), 10-fold (orange), and >10-fold (red) of observed clearance. Closed circles represent Ro5 compounds; open circles represent bRo5 compounds. The solid line indicates unity, while dashed and dotted lines indicate 3-fold and 10-fold deviations, respectively. The blue line represents the linear fit. TruVivo demonstrates improved predictive accuracy, particularly for low-clearance and non-Ro5 compounds, consistent with enhanced metabolic activity and extended assay windows. Figure reproduced from portions of Figure 3 of Kukla *et al*. with permission under an open access license.[1]

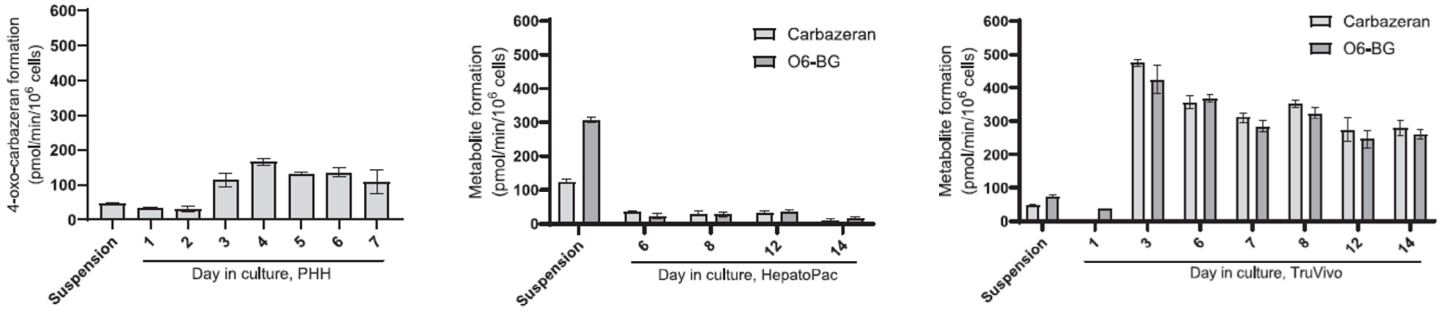

Figure 3 (Below). **Aldehyde oxidase (AO) activity over time in three hepatic model systems**—standard plated monocultures (LifeNet Health lot 1921756), HepatoPac (BioIVT lot LLT), and TruVivo (LifeNet Health lot 1921756)—adapted from Byer-Alcorace *et al*. AO activity in plated monocultures and HepatoPac declined rapidly, with measurable activity largely lost within the first 24–48 hours. In contrast, AO activity in TruVivo increased approximately 10-fold by Day 3 and remained high and stable through Day 14, as indicated by continuous formation of AO-specific metabolites. Figure reproduced from portions of Figure 2 of Byer-Alcorace *et al*. with permission under an open access license.[2]

Why Researchers Choose TruVivo

Researcher Need	TruVivo Advantage
Extended Functional Lifespan (14+ days)	TruVivo enables long incubations and reliable assessment of low-clearance compounds[1,2]
Physiologically-relevant Microenvironment	Self-assembled primary human hepatocyte, stromal, and endothelial cell interactions maintain metabolic fidelity and long-term enzyme function[2,3]
Improved Reproducibility	Supports consistent screening with low donor-to-donor variability compared to traditional models.[1]
Reliable Turnover for Stable Compounds	Only 4% insufficient turnover in TruVivo vs. 40% in suspension hepatocytes.[1]
High Prediction Accuracy	3-fold accuracy for majority of 50-compound panel.[1] 2-fold accuracy for AO-selective substrates.[2]
Robust AO Activity	High AO activity, accurate AO-dependent CL_h and $f_{m,AO}$.[2]

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Research Applications

- Quantify intrinsic and blood clearance for slowly metabolized compounds that are not amenable to short-term assays, enabling **low-turnover clearance prediction**.
- **Characterize aldehyde oxidase (AO)–mediated metabolism**, including estimation of $f_{m,AO}$, to support prediction of *in vivo* pharmacokinetics and drug–drug interaction risk.
- Generate human-relevant Phase I and II clearance data to support **IVIVE and PBPK modeling** for improved prediction of human pharmacokinetics, dosing, and drug–drug interaction scenarios, including time-dependent clearance.
- **Elucidate drug clearance mechanisms** by defining contributions of CYP, AO, and UGT pathways, enabling identification of slowly formed metabolites and assessment of transporter–enzyme interactions.

References

1. **Kukla D.A., Schulz Pauly J.A., Lesniak P.R., et al. (2024).** *Clearance prediction with three novel plated human hepatocyte models compared to conventional suspension assays: Assessment with 50 compounds and multiple donors.* Drug Metabolism and Disposition 53 (2025) DOI: 10.1016/j.dmd.2024.100032
2. **Byer-Alcorace A., Thomas C., Taub M.E., Piekos S. (2025).** *Improved clearance predictions for aldehyde oxidase substrates using a novel triculture human hepatocyte model.* Drug Metabolism and Disposition 53 (2025) DOI: 10.1016/j.dmd.2025.100051
3. **Weaver, J., Odanga J., Wolf, K., et al. (2023).** *The morphology, functionality, and longevity of a novel all human hepatic cell-based tri-culture system.* Toxicology *In Vitro*, volume 86 (2023). DOI: 10.1016/j.tiv.2022.105504