A Cross-Laboratory Comparison of Caco-2 and Human Intestinal Drug Transporter Protein Abundances
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Introduction: Numerous laboratories utilising a diverse range of techniques are quantifying the absolute abundance of drug transporter proteins by Quantitative Targeted Absolute Proteomic (QTAP) strategies in mammalian tissues and in vitro cell systems. Ten-fold differences in absolute abundances have been observed for specific transporter isoforms in non-matched samples between laboratories, for example, hepatic OATP1B1,2. Ascertainment if differences in abundances are derived from intrinsic biological variability, or variability associated with assay-specific techniques, and/or specific data analysis within each laboratory, are crucial for generating robust PBPK models that reflect in vivo abundances. Therefore, a multi-centre study evaluating the consistency and comparability of the preparation steps and analytic outcome has been advocated. Caco-2 and human intestinal membrane fractions were prepared. This study then assessed the comparability in absolute transporter abundances for 3 proteins; Sodium/Potassium-ATPase (Na/K-ATPase), P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) in membrane fractions, within 2 independent laboratories, The University of Manchester (UoM), Manchester, UK and Bertin Pharma (BPh), Orleans, France.

Methods: Caco-2 cells (ATCC-HTB-37) passage 25-35 (n=8) or passage 111 were grown for 10, 21 and, 29 days on 44-cm² Transwell filters (0.4 μm pore size).

Macroposiately normal human distal ileum (DI, n=3) and distal ileum (DI, n=1) were obtained immediately after resection from patients undergoing elective surgery at Salford Royal Hospital under consent (REC 12/NW 0306). Caco-2 cells were harvested from filters and fresh human intestinal enterocytes harvested by elution using an EDTA-chelation method, underwent a differential centrifugation procedure to obtain total or plasma membrane fractions and were stored at -80°C. Protein content was subsequently determined by BCA assay. Membrane proteins (typically >50 μg) were either shipped on dry ice to BPh or retained for analysis at UoM.

The University of Manchester

<table>
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<tr>
<th>Peptide Selection</th>
<th>Standard Generation</th>
<th>Selected Peptides</th>
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<th>LC-MS/MS</th>
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<tr>
<td>In Silico – UoM</td>
<td>-QconCAT program</td>
<td>Na/K-ATPase, P-gp, BCRP</td>
<td>Deoxocholate denaturation, Lys-C + trypsin digest</td>
<td>Nano flow LC – nanoAcuity (Waters) with TSQ Vantage (Thermo), selected reaction monitoring</td>
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Bertin Pharma

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<td>In Silico - Tohoku University – Professor Tetsuya Terasaki</td>
<td>-Absolute Quantification (AQUA)</td>
<td>Na/K-ATPase, P-gp, BCRP</td>
<td>MS2Plex-based process including trypsin-based digest</td>
<td>Normal flow LC – Flexar LC (Perkin Elmer) with API5500 (AB Sciex), selected reaction monitoring</td>
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The native to standard peak area under the curve ratio for ≥ 2 selected transitions for each peptide was used to calculate absolute protein abundances in femtomol (fmol) per μg (fmol/μg) membrane protein.

References:
1. Prasad et al, 2014, DMD, 42, 78-88

Results: Quality control – Peptide linearity, precision (CV≤15%) and quantification limits (LLOQ) were determined – LLOQ BPh-0.125 fmol/μg, UoM-0.2 fmol/μg

A – Na/K-ATPase

B – P-gp

C – BCRP

Discussion & Conclusion:
Overall, UoM quantification for P-gp was significantly higher and correlated with BPh. Peptide selection, digestion or LC-MS/MS conditions may lead to differences in quantitation.

No mean abundance or sample correlation differences exist for Na/K-ATPase or BCRP between laboratories.

Identifying & accounting for methodological bias is crucial when incorporating data into PBPK models. However, the relevance of any differences in abundance quantification on the success of model outcomes requires ascertaining. Characterising protein losses during preparation is advocated with further across-laboratory studies on matched samples required.

Figure 1. A-C – Absolute protein abundances for Na/K-ATPase, P-gp and BCRP determined by BPh (black bars) and UoM (white bars) in Caco-2 cells and human intestines. BLQ = below limit of quantification. * p < .05. Test above bars indicates Caco-2 growth age, total or plasma membrane (TM/PM) and HP is high passage Caco-2. D-F – Plots showing correlation of Na/K-ATPase, P-gp and BCRP absolute abundances between BPh and UoM. Diamonds denote human (white) and Caco-2 cells (black).