Complementary in vitro tools to investigate renal drug transport
V. Juhász1, E. Beéry1, Zs. Gáborik1, G. Imre1, C. Brown2, J. Zolnerciks3 and B. Oosterhuis1
1SOLVO Biotechnology, Budärs, Hungary, 2Institute for Cell & Molecular Bioscience Medical School Newcastle University, Newcastle, United Kingdom, 3SOLVO Biotechnology USA, Seattle, WA

Introduction
Renal excretion is an important pathway for the elimination of endogenous and xenobiotic substances. A wide range of efflux and uptake transporters are expressed in the renal epithelial cells to regulate the excretion and the reabsorption of various kinds of organic anions, cations, peptides and nucleosides. The purpose of this work is to compare two in vitro systems for investigating renal handling. The primary proximal tubule cell (PTC) monolayer assay (established by Dr Colin Brown, Newcastle University, UK) serves as a holistic approach, while double transfected monolayer assays could complete the results of PTC monolayer with functional characteristics, such as substrate specificity and transport mechanisms involved in the renal elimination.

Material and Methods
PTCs were isolated from human kidney less than 18 hours ex vivo as follows: 1) kidney decapsulated, cortex dissected and finely chopped; 2) 2 hour collagenase digest in isolation medium at 37°C; 3) the heterogeneous cell population was passed through 40 µm sieve, then separated by density centrifugation; 4) The tubular cell layer was extracted and cultured on transwell inserts. Unlike other primary kidney cell-based models, the PTC model retains the expressions and the functionality of the kidney transporters.

Experiments were performed when human PTC monolayers had a TEER of 80 Ocm² or greater. Uni-directional fluxes of several probe substrates in either the apical to basolateral (Papp) or basolateral to apical (Papp) direction were measured.

The double transfected cell lines (MDCKII-OCT2/MATE1, MDCKII-OCT2/MATE2-K, MDCKII-OAT1/BCRP and MDCKII-OAT3/BCRP) were developed by SOLVO Biotechnology. The double transfected and the corresponding control cells (parental MDCKII and single transfected transporter expressing MDCKII cells) were plated on 24-transwell plates, and bidirectional transport of several probe substrates were measured after 5 days.

Conclusion
- The vectorial transport of several key prototypic substance (Figure 1) demonstrated that the function of the PTC monolayers is retained to a considerable degree. Consequently, PTC monolayer provides a physiologically relevant and holistic in vitro model of renal drug handling.
- Double transfected monolayer assays, on the other hand, provide in vitro tools to investigate the specific transporters that could contribute to the active elimination of either organic cationic (Figure 2: MDCKII-OCT2/MATE2-K and Figure 3: MDCKII-OCT2/MATE1) and anionic (Figure 4: MDCKII-OAT1/BCRP and Figure 5: MDCKII-OAT3/BCRP) compounds.
- Altogether, double transfected cell lines with key renal transporters paired according to their overlapping substrate specificities, partnered with the PTC monolayers will give a powerful holistic insight into the impact of transporters on renal clearance.