

CACO-2 Monolayer Assays at SOLVO

Differentiated Caco-2 cells (a human colon carcinoma cell line) express a wide range of transporter proteins on its cell membranes (Siissalo S et al. 2007.) similar to those of intestinal endothelium cells (Calcagno AM et al. 2006.). This makes Caco-2 cells ideal for intestinal absorption simulations. In fact, in the last decade, the utilization of Caco-2 cells has become an industry standard for the investigation of intestinal absorption, permeability and drug-drug interactions (DDIs) (Oh DM et al. 2002.).

The Caco-2 monolayer efflux assay is designed to model the net transport events of an important fluid compartment barrier in the organism. This method utilizes a polarized Caco-2 cell layer grown on a supportive membrane surface that separates the two compartments (Figure 1). The unidirectional flux of the test article (TA) is determined by applying the TA to either the apical or to the basolateral side of the cell layer and monitoring the time resolved redistribution of TA between the two compartments. The vectorial transport ratio is determined by applying bidirectional measurements [apical-to-basolateral (A-B) and basolateral-to-apical (B-A)].

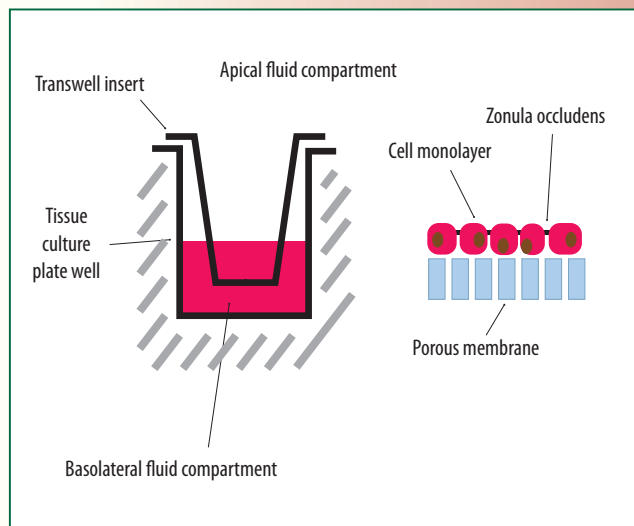


Figure1: Schematic representation of the basic principles of the monolayer assay system.

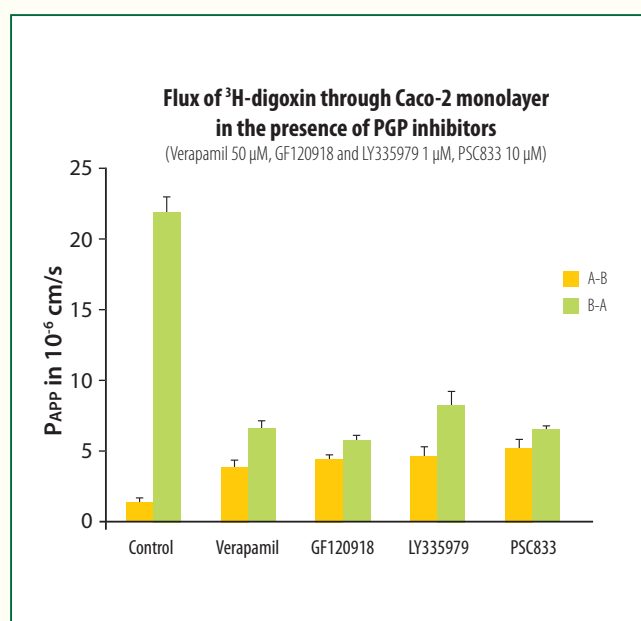


Figure 2: Flux of ³H-digoxin through Caco-2 monolayer in the presence of verapamil, GF120818, LY335979 and PSC833.

In general, a BA/AB ratio higher than 2 or lower than 0.5 indicates the contribution of an active transport process to the net flux of a compound. In the absence of such transport processes this ratio is around 1. A vectorial transport ratio of 1 does not indicate the absence of active transport. For example, in the case of highly permeable compounds the overall contribution of the active transport process to the net flux might be undetectable. Yet, these compounds might interfere with the transport of other compounds that might result in a clinical drug-drug interaction. This can be assayed by measuring the modification of the vectorial transport ratio of a reporter compound that is known to be affected by some active transport process.

A typical example of an indirect assay to estimate P-gp mediated drug-drug interactions is to measure the effect of the TA on the flux of the reporter substrate, ³H-digoxin (Figure 2).

