P01-16
In vitro permeability assay using an epithelial model of Caco-2/HT29-MTX/Raji-B cells: enhancer aspects of a papain-cyclodextrin complex

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The oral route is one of the main routes for administration of drugs, however, the gastrointestinal tract is a hostile environment due to pH variation, presence of several digestive enzymes and the intestinal barrier that undermines the permeation of drugs. The aim of this study was to evaluate the ability of papain complexed with β-cyclodextrin to enhancer the permeation of furosemide, as a model drug, in a triple co-culture of Caco-2, HT29-MTX and Raji cells. Papain and the papain-β-cyclodextrin complex were evaluated at 0.3, 0.7 and 1.0 μM and the biophysical integrity of the cell layer was evaluated by RET (Transepitelial Electrical Resistance) at 0, 4, 24, 48 and 72 hours. The epithelium was also stained using DAPI and Alexa Fluor™ 488 Phalloidin. HPLC was employed to quantification of furosemide. The RET results at initial time for all the samples and control were in a range of 267.63 to 318.28 Ω^*cm^2 and after 72 h this values were raised to a range of 365.14 to 492.64 Ω^*cm^2. There was a decrease of RET after samples' application, nevertheless, the results showed that the epithelium presents a recovery, proportional to the time of cell replication, and that this recovery occurs in all samples tested with no significant statistical difference. The RET recovery implies that papain, complexed or not, was not able to kill the cells, corroborating the hypothesis that the action mechanism is the disruption of the tight junction. In addition, the triple co-culture presents a higher resistance to papain action, in comparison with the Caco-2 monolayer assays, emphasizing the importance of testing new drugs, potential candidates for oral formulations, in epitheliums that faithfully mimics what actually happens in *in vivo* systems. The fluorescent microscopy observation of the cells stained with DAPI and the junctions stained with Alexa Fluor™ 488 Phalloidin, showed that co-culture exhibits microvilli inherent to the intestinal tissue. The results obtained in the triple co-culture model bi-directional transport experiments confirmed the significant increase in furosemide transport indicating the importance of the paracellular route. In conclusion, the triple co-culture model was successfully standardized and papain complexed with β-cyclodextrin acts probably over the tight junctions enhancing the permeation of furosemide.

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P01-17
Assay ready frozen THP-1 cells can be used like a reagent in a human Cell Line Activation Test (h-CLAT) to measure the skin sensitizing potential of chemicals

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Recently a cell based *in vitro* model has have been approved by the OECD (Test N° 442E) to assess the skin sensitizing hazard of chemicals. One of the tests applied is the human Cell Line Activation Test (h-CLAT) which uses monocytic THP-1 cells as a surrogate for dendritic cells. These cells express CD86 and CD54 upon activation through sensitizing molecules at subtoxic concentrations.

A major obstacle of the assay is the cultivation of the THP-1 cells. The concentration rage at which skin sensitization can be measured without significantly reducing viability, is very tight. A healthy and highly viable culture of THP-1 cells is therefore of the essence. THP-1 are known to recover badly from suboptimal cultivation or cryopreservation. It usually takes at least a week of intensive care until the cells regain an acceptable viability. Because the overall fitness of the cells has a significant impact on their sensitivity to sensitizers, reproducibility of the h-CLAT very much depends on the cell culture quality and is therefore difficult to control.

We here present an optimized cryopreservation protocol for THP-1 cells. These assay ready cells recover from cryostock at a high and stable viability of greater than 90%. They can be applied in a h-CLAT skin sensitization testing immediately after resuscitation. No prior cultivation or passaging is required which eliminates the cell culture factor from the assay. By applying different reference substances like NiSO4 or DNCB we demonstrate the assay ready frozen THP-1 are equally susceptible to skin sensitizers like cells from a continuously passaged maintenance culture and provide a better reproducibility.

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P01-18
Human *In Vitro* models for respiratory toxicology: evaluation of goblet cell hyperplasia, mucus production, and ciliary beating assays

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Robust non-animal models and assays for pulmonary toxicology are required to make competent product development and risk assessments for new materials requiring toxicity testing. Three *in vitro* assays (goblet cell hyperplasia [GCH], ciliary beat frequency [CBF], and MUC5AC quantitation) were evaluated for performance and reproducibility. To assess these assays, 6 laboratories contributed data using a common protocol utilizing IL-13 as an inducer of adverse mucociliary-relevant tissue changes. MatTek EpiAirway™ and Epithelix MucilAir™ 3D tissue models were used to evaluate endpoints using histology for GCH, software-based applications, Cilia FA and SAVA, for CBF, and ELISA assay for MUC5AC. Continuous 10 ng/mL IL-13 (GCH, MUC5AC) exposures or one hour 10 μM procaterol (CBF) exposures prior to day 7 and 14 time-points were included as positive controls. Quality control endpoints (e.g. adenylate kinase tissue content and trans-epithelial electrical resistance) were also evaluated. Multi-fold increases (ranging from 2.6 to 33-fold, and 1.5 to 238-fold) in MUC5AC-stained goblet cells were measured in both tissue models after exposure with IL-13 after 7 and 14 days induction, respectively. For CBF, procaterol caused a significant increase, and IL-13 elicited a significant decrease as expected. However, the MUC5AC ELISA did not yield consistent results when frozen apical rinse samples were thawed and assayed. These results suggest these non-animal test systems may provide consistent, human-relevant data corresponding to key events involved in respiratory disease. A streamlined protocol using these controls will be applied toward additional testing. These assays, utilized in a pragmatic manner with other *in vitro* assays have