Validation of the Vitrocell® 48 2.0+ 24-well mammalian module for assessing cigarettes and eHTPs: Evaluation of dilution airflow, dose resolution and 3D tissue suitability

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Abstract

The use of 3D *in vitro* airway tissue models that closely mimic the physiological architecture and microenvironment of the human respiratory system is critically important when studying aerosol exposure. Presence of a pseudostratified mucociliary epithelium and mucus secreting goblet cells help to provide a more accurate representation of the deposition, dissolution and clearance patterns of inhaled aerosols during human exposure.

The objective of this study was to validate the Vitrocell® 48 2.0+ 24-well module by quantifying delivered dose across a range of dilutions from both reference cigarettes and electronically heated tobacco products (eHTPs). Variability and repeatability were assessed through dosimetric assessment of nicotine, TPM fluorescence and free glycerol. The suitability of Mucilair™ 3D tissues within the system was also tested; viability and cytotoxicity were assessed via WST-8 reduction and Lactic dehydrogenase release; physiological changes were quantified by TEER and CBF and inflammation was investigated by measurement of cytokine levels.

Results of dosimetric assessment showed no statistical difference between replicates within dose on all but the lowest dilution (0.5 L/min 1R6F, 0 L/min eHTP) and good dose resolution at higher doses, with a loss of resolution noted as dilution rates increase. Cytotoxicity results showed high concordance with historical data generated in Vitrocell® 24/4 module format (p=0.9296).

In conclusion, the validation of the Vitrocell® HTP 2.0+ 12 well mammalian module demonstrated reliable performance for assessing cigarettes and eHTPs. The study findings revealed low variation within dilution airflow, overall good dose resolution and supports the use of this module in relevant toxicological investigations and risk assessments.

Methods

Aerosol from each test article was generated using a Vitrocell® VC10® smoking robot and diluted to the desired concentrations via mass flow controllers prior to entry into the HTP 2.0+ module. Liquid traps were placed at each position within the module and aerosol was directed over the trap via restricted flow negative pressure at a rate of 5 mL/min. Exposure time was approximately 1 hour, rounded to the nearest whole consumable. Liquid traps mirrored expected exposure conditions through the use of manufactured stainless steel transwells, and solvent volume was calculated to ensure apical surface was 2 mm below the trumpet.

Upon exposure completion, solvent traps for HTPs were analysed for glycerol (sigma F6428) and nicotine (LC-MS/MS), and traps for 1R6F analysed for TPM fluorescence and nicotine (LC-MS/MS). The linear range for nicotine quantification was 0.08 to 50 µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were 0.026 and 0.08 µg/mL, respectively.

Mucilair™ Suitability was confirmed via 64-minute 1R6F ISO 20778 exposure at dilution rates of 10, 8, 6, 5, 4, 3 and 2 L/min. Relative survival as a percentage of air control was quantified by colorimetric assessment of WST-8 tetrazolium salt reduction to formazan (460nm) 24-hour post-exposure.

Results

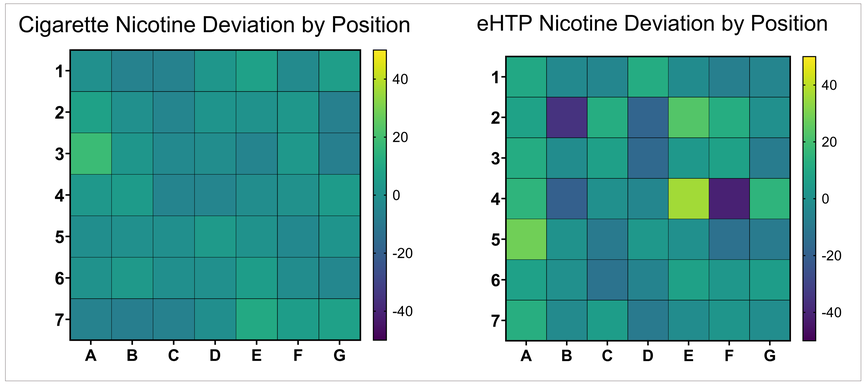


Figure 1. Heatmap showing % deviation of nicotine within each row for 1R6F and a commercially available eHTP, respectively.

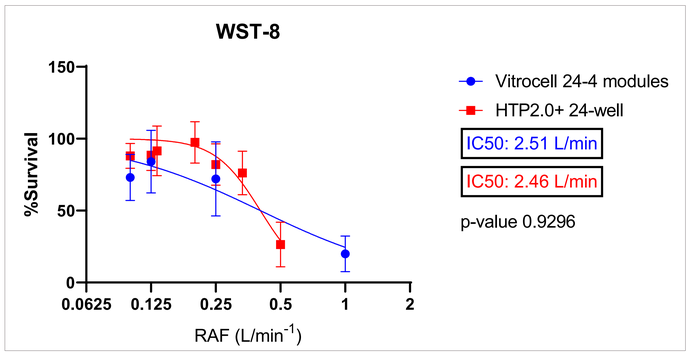


Figure 2. % Survival comparing Vitrocell 24-4 and HTP 2.0+ 24 well module formats.

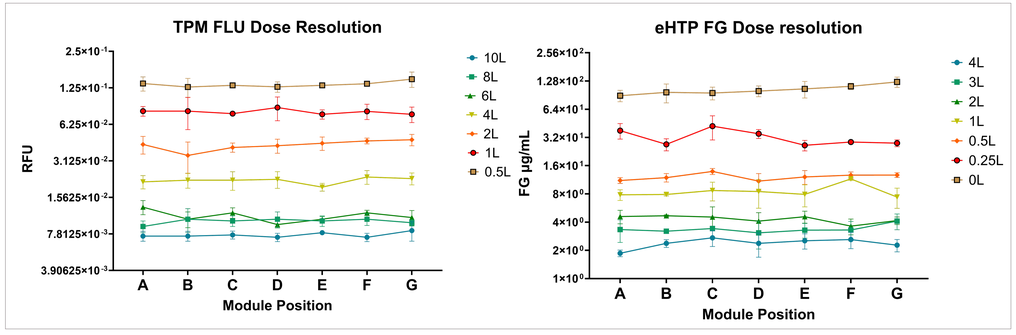
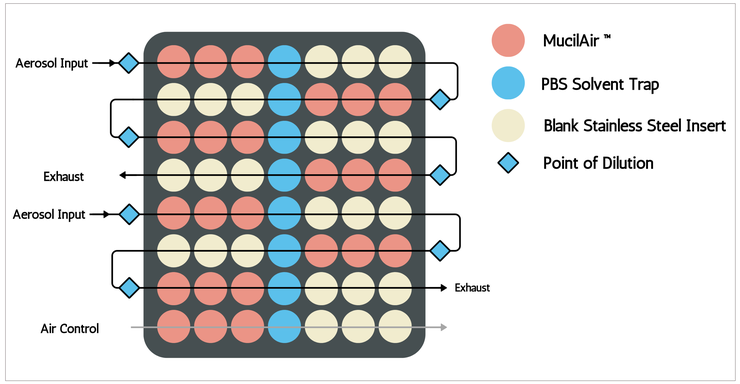


Figure 3. Dose resolution plot utilising dosimetric assays of TPM fluorescence and free glycerol concentration, respectively.

Exposure layout



Diagrammatic representation of 64-minute 1R6F ISO 20778 exposure. Aerosol input was limited to two to maintain an exhaust duration of 8 seconds and PBS solvent traps were included for dosimetric assessment. Stainless steel inserts were placed in blank wells to reduce any upstream effects.

Conclusions

This validation study on the Vitrocell® 48 2.0+ 24-well module has successfully demonstrated its reliability for assessing both conventional cigarettes and eHTPs. Notably the module exhibited low variation within dilution airflow, with statistical differences only observed at 0 L/min dilution and no positional bias shown across both test articles.

The system also displayed good dose resolution, particularly at higher doses, although it is worth noting that this resolution tended to decrease at higher dilution airflows. This characteristic allows for precise dose-response assessments, especially in more concentrated and physiologically relevant exposure scenarios. Furthermore, the cytotoxicity results showed high concordance with historical data generated with the Vitrocell® 24/4 module format, with a p-value of 0.9296 and inline IC50 values indicating strong reliability and comparability with already established methods.

The successful integration of Mucilair™ tissues along with the increased number of both replicates and dilutions allow much greater insight into more physiologically relevant exposure conditions, allowing the system to mimic real-world inhalation scenarios and provide more accurate insights into the effects of aerosol exposure.

In conclusion these findings collectively support the use of the Vitrocell® 48 2.0+ 24-well module in the toxicological investigations and risk assessments for both cigarettes and eHTPs. The system offers a reliable and physiologically relevant platform for studying aerosol exposure effects, representing an important step forward in inhalation toxicology research.

CORESTA Congress; October 13-17, 2024; Edinburgh, Scotland