Validation of the Vitrocell® HTP 2.0+ 12-well mammalian module for assessing cigarettes, ENDS and eHTPs: Evaluation of dilution airflow, dose resolution and dose repeatability

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Abstract

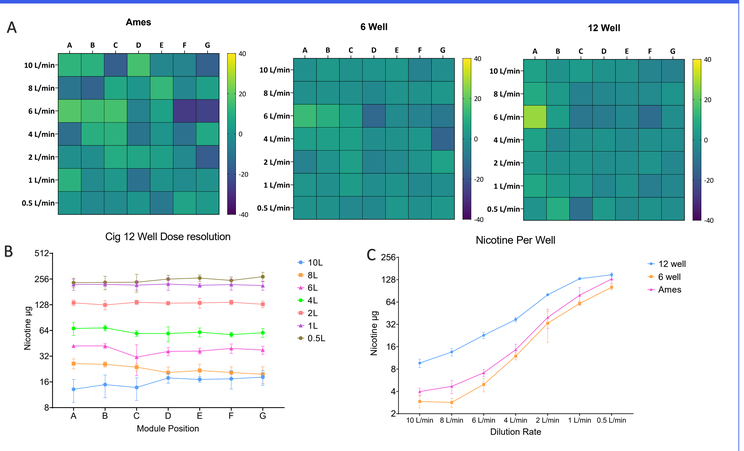
The present study aimed to validate the performance of the Vitrocell® HTP 2.0+ 12-well mammalian module for assessing three different test articles: cigarettes, electronic nicotine delivery systems (ENDS) and electronic tobacco heated products (eHTPs). Specifically, we investigated the variation within dilution airflow, dose resolution and dose repeatability to ensure the reliability of the experimental setup. To evaluate dilution airflow, measurements were conducted across all test articles, revealing consistently low variation, except for the lowest dilution rates where higher variability was observed. Overall, the dilution airflow showed satisfactory performance, indicating its suitability for use in the experimental system. Dose resolution, a critical parameter for accurate assessment, was assessed for all test articles. The results demonstrated good dose resolution in general, although ENDS exhibited the lowest resolving power among the three tested products. This finding emphasizes the need for careful consideration when evaluating ENDS to ensure accurate dosing and subsequent analysis. Furthermore, the experimental dose repeatability was examined. Notably, the repeatability was highest on test articles that required multiple products per dose, cigarettes and eHTPs, indicating the importance of accounting for pod-to-pod variability when planning exposures using ENDS. In conclusion, the validation of the Vitrocell® HTP 2.0+ 12-well mammalian module demonstrated reliable performance for assessing cigarettes, ENDS and eHTPs. The study findings revealed low variation within dilution airflow, overall good dose resolution and highlighted the significance of considering pod-to-pod variability for ENDS exposures. These findings contribute to the accurate evaluation of aerosol exposures and support 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 each HTP2.0+ module type. 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 5ml/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™ or 35-mm Petri dishes, solvent volume was calculated to ensure apical surface was 2mm below the trumpet. Upon exposure completion solvent traps were analysed for glycerol (sigma F6428) 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.

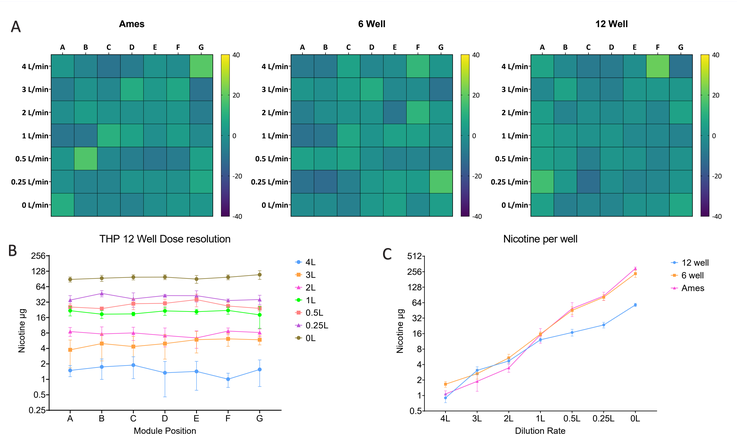
Results

Combustible Cigarettes



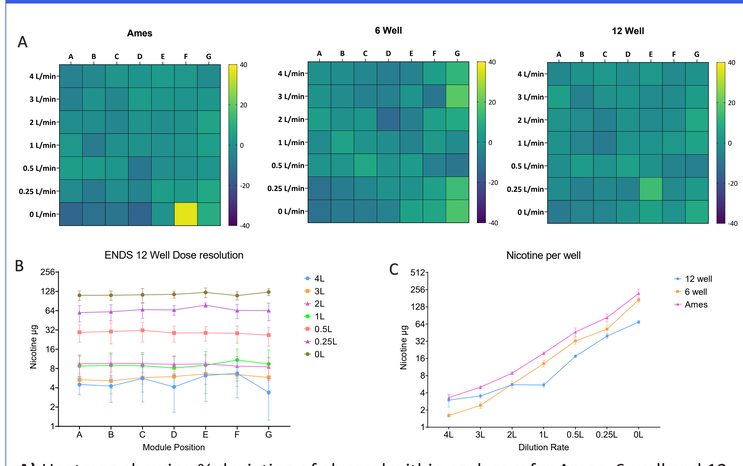
A) Heatmap showing % deviation of glycerol within each row for Ames, 6-well and 12-well module types, respectively. **B)** Representative dose resolution plot (only 12-well data shown). **C)** Plot of N=3 exposures showing repeatability and nicotine deposition.

Tobacco Heated Products



A) Heatmap showing % deviation of glycerol within each row for Ames, 6-well and 12-well module types, respectively. **B)** Representative dose resolution plot (only 12-well data shown). **C)** Plot of N=3 exposures showing repeatability and nicotine deposition.

Electronic Nicotine Delivery Systems



A) Heatmap showing % deviation of glycerol within each row for Ames, 6-well and 12-well module types, respectively. B) Representative dose resolution plot (only 12-well data shown). **C)** Plot of N=3 exposures showing repeatability and nicotine deposition.

- Within independent exposures, under specified puffing parameters, freshly generated whole aerosol from 1R6F, HTP and ENDS were delivered consistently within all three module types: Ames, 6-well and 12-well using nicotine and the dosimetry marker.
- Overall, the majority of positions were within the manufacturer's specified ranged of ±15% the overall airflow mean. Ames modules with ENDS rep F with undiluted is a clear outlier in all independent experiments. No technical deviations could be observed. Further work may be required to determine if this results in a biological difference.
- Nicotine deposition increases in a concentration manner across all modules and product types.
- There is a clear difference in dose resolution for 1R6F and HTP products. There is less definition between the higher airflows for ENDS between 4 and 1 L/min. This could be due to lower deposition rates.
- These results show that exposures utilizing the Vitrocell®HTP2.0+ demonstrate consistent and reproducible delivery of whole smoke and aerosol. The few outliers present do indicate the necessity of including dosimetry measurements in every exposure.

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