

Figure E1a. Condensation system used for production of e-cigarette vapour condensate. Five tracheal suction traps, connected in series and sealed with parafilm were partially submerged in methanol, surrounded by dry ice, with the sixth connected directly to the device. Airflow was created by attachment of a vacuum source. A 3s on/30s off puffing system was employed to simulate puffing a user would perform.



Figure E1.b Picture of Kanger e-cig used for production of ECVC/nfECVC.

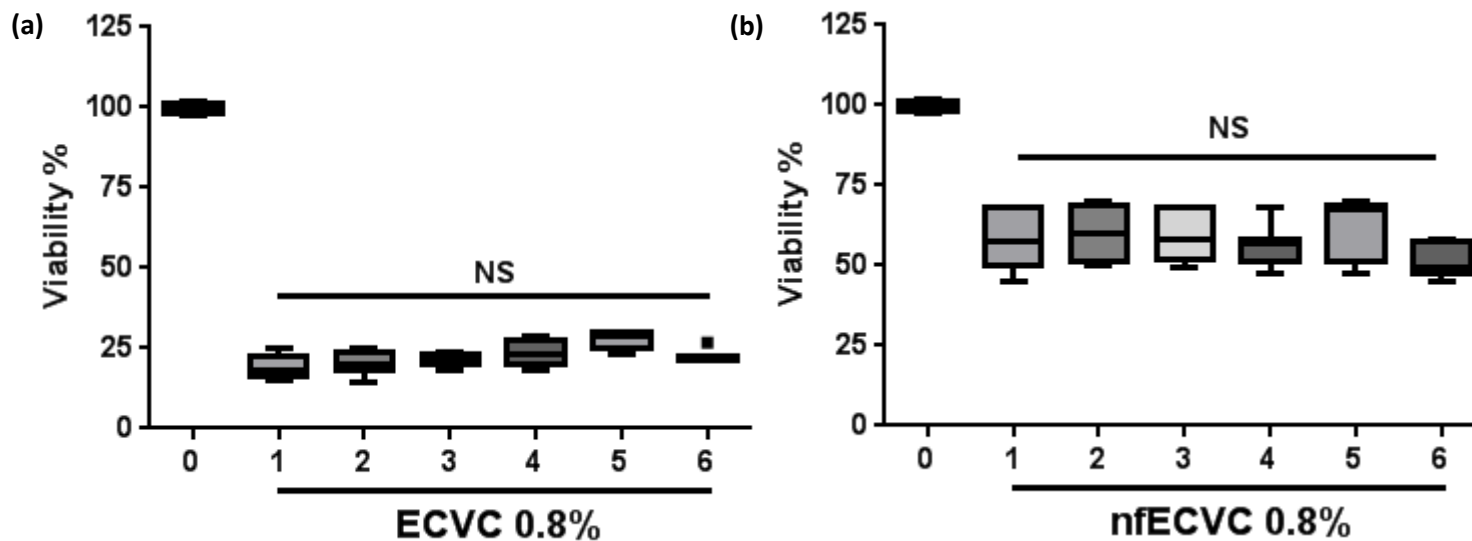


Figure E2. Comparison of fresh preparations of ECVC/nfECVC as measured by cytotoxicity on THP-1 macrophages. Viability was assessed by 4h incubation with cell titre aqueous assay following 24hr exposure to each fresh preparation of condensate. Graphs presented as median with interquartile range of 8 independent experiments. The central horizontal line on each box plot represents the median, the upper and lower horizontal lines represent the first (Q1) and third (Q3) quartiles, respectively, and the vertical lines represent the range of values within the limits $Q1 - 1.5(Q3 - Q1)$ and $Q3 + 1.5(Q3 - Q1)$.

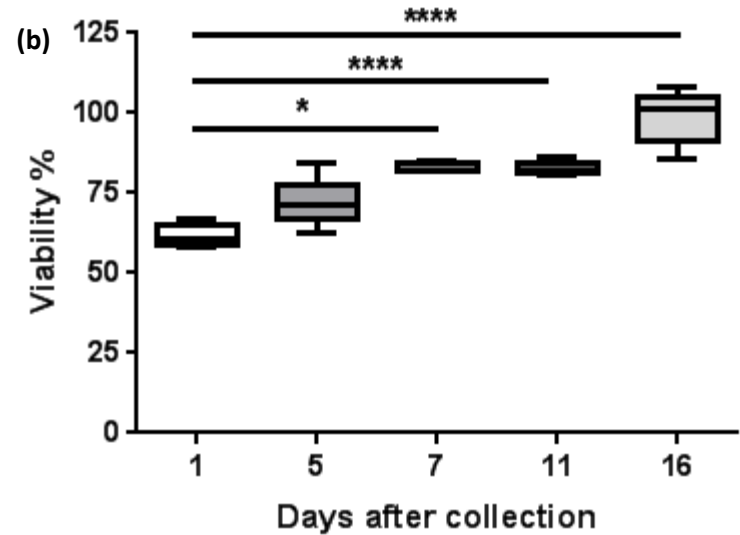
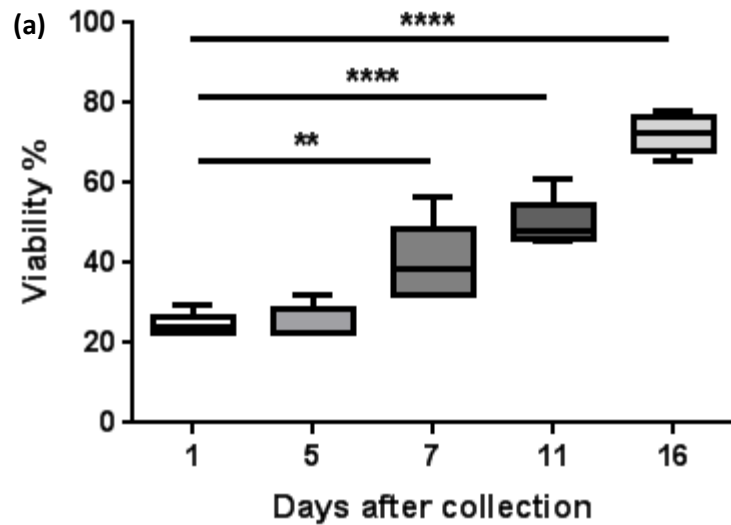


Figure E3. Assessment of variation over time in the cytotoxic potential of ECVC/nfECVC using THP-1 macrophages. Viability was assessed by 4h incubation with cell titre aqueous assay following 24hr exposure to each fresh preparation of condensate. Graphs presented as median with interquartile range of 8 independent experiments. The central horizontal line on each box plot represents the median, the upper and lower horizontal lines represent the first (Q1) and third (Q3) quartiles, respectively, and the vertical lines represent the range of values within the limits $Q1 - 1.5(Q3 - Q1)$ and $Q3 - 1.5(Q3 - Q1)$.

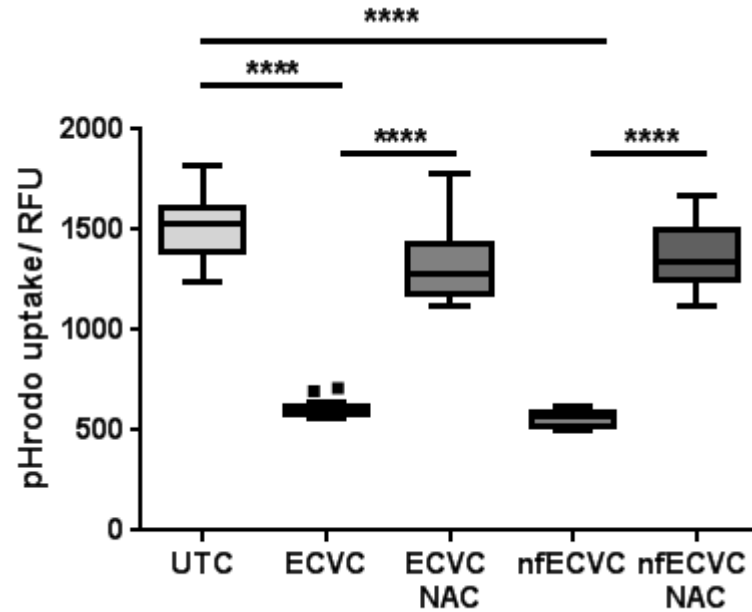


Figure E4. Functional effects of ECVC/nfECVC exposure to THP-1 macrophages, exposed to 0.5% ECVC/nfECVC for 6 hours, following which uptake of *S.aureus* pHrodo bioparticles was assessed. Graphs presented as median with interquartile range of 8 independent experiments. The central horizontal line on each box plot represents the median, the upper and lower horizontal lines represent the first (Q1) and third (Q3) quartiles, respectively, and the vertical lines represent the range of values within the limits $Q1 - 1.5 (Q3 - Q1)$ and $Q3 - 1.5 (Q3 - Q1)$.

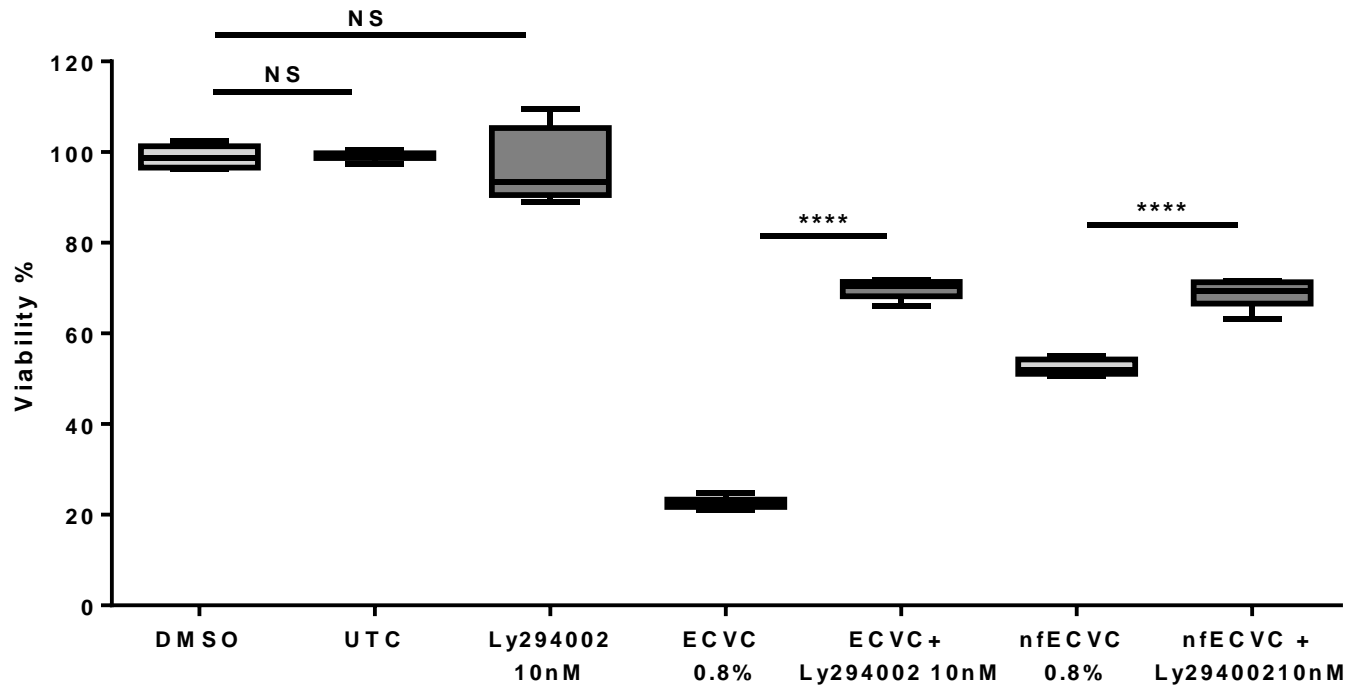


Figure E5. Effect of ECVC/nfECVC and PI3K inhibitor Ly294002 on alveolar macrophage viability. Viability was assessed by 4h incubation with cell titre aqueous assay following 24hr exposure to Pan-PI3K inhibitor Ly292004 and challenged with ECVC or nfECVC (0.8%). Graphs presented as median with interquartile range of 6 independent experiments. The central horizontal line on each box plot represents the median, the upper and lower horizontal lines represent the first (Q1) and third (Q3) quartiles, respectively, and the vertical lines represent the range of values within the limits Q1 - 1.5 (Q3 - Q1) and Q3 - 1.5 (Q3 - Q1).

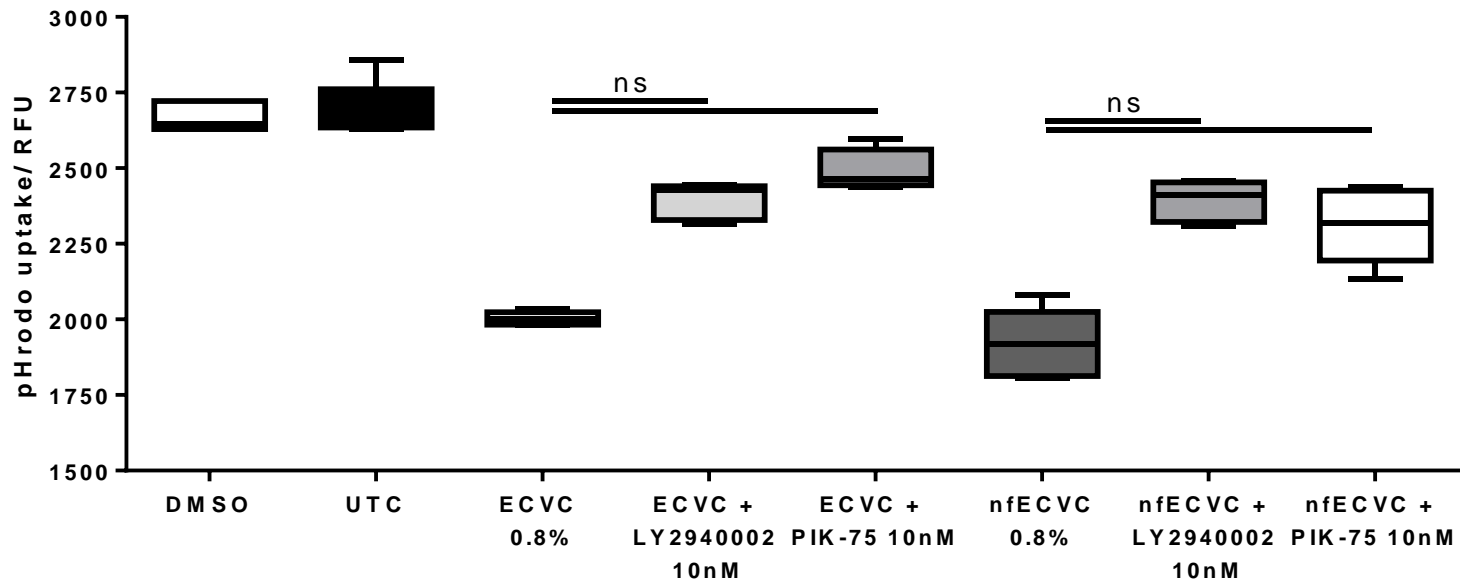


Figure E6. Functional effects of ECVC/nfECVC exposure to THP-1 macrophages, exposed to ECVC or nfECVC (0.8%), with and without Pan-PI3K inhibitor Ly292004 and isoform selective inhibitor PIK-75 for 6hrs. Uptake of *E.coli* pHrodo bioparticles was then assessed. Graphs presented as median with interquartile range of 8 independent experiments. The central horizontal line on each box plot represents the median, the upper and lower horizontal lines represent the first (Q1) and third (Q3) quartiles, respectively, and the vertical lines represent the range of values within the limits Q1 - 1.5 (Q3 - Q1) and Q3 - 1.5 (Q3 - Q1).