Transcript of Whole Smoke Exposure video

The whole smoke exposure system comprises two main parts – a smoking machine, and whole smoke chambers, designed by and patented by British American Tobacco.

The whole smoke system can use any type of cultured cell providing it can be supported on porous Transwell® membranes.

Transwells® are removed from their culture plate and excess media is poured off. The Transwells are then individually placed in the smoke exposure chamber.

Any remaining media is removed from the Transwell using a pipette, with care taken not to disrupt the cultured cells or damage the porous membrane.

Removal of any residual media ensures that cells are evenly dosed during exposure, and the air-liquid interface is maintained. Once all of the transwells are placed into the chamber, the lid is secured and the bolts are tightened. At this point the chamber is sealed and sterile and can be removed from the hood and placed in the whole smoke set-up.

Cigarette smoke is generated using a Borgwaldt RM20s smoke diluter. A 2 second, 35ml puff is taken every minute. Smoke is diluted by a syringe based system, which allows a broad range of dilutions to be achieved. The smoke machine has eight syringes, capable of eight different dilutions per cigarette, or one dilution per 8 cigarettes. This offers flexibility in experimental design.

The exposure chamber houses the cells at an air-liquid interface, which is a more physiologically accurate representation of lung epithelium compared to submerged culture models.

The sealed exposure chamber is placed in an incubator pre-heated to 37℃, and attached to the whole smoke system via a series of connecting tubes.

Contact between the media and the basal surface of the cells is regulated by dual peristaltic pumps without flooding the cell monolayer or disrupting the air-liquid interface. Media flow is unidirectional to avoid build up of smoke constituents, which may affect exposure conditions.

Cells are fed basally and media is delivered to them through the ‘media in’ port, while the ‘media out’ port removes media and ensures that the air-liquid interface is maintained.

Diluted mainstream cigarette smoke is exhausted directly from the smoking machine via the dilution syringes, and then delivered to the cells apically through the ’smoke in’ port.

The distribution plate ensures that smoke is dispersed evenly throughout the chamber. Inside the chamber, smoke is diffused via ‘Brownian motion’, which is the random movement of particles suspended in liquid or gas, caused by collisions with molecules in the surrounding environment.

Smoke is expelled from the chamber via the ‘smoke out’ port by a passive process when the next diluted puff is delivered by the smoking machine.

The flexibility of the exposure chamber to accept a variety of cell types and co cultures, combined with an adaptable smoking machine, can provide complex and innovative experimental designs.

Following exposure of cells to whole smoke we can investigate cellular responses such as protein expression, gene expression, DNA damage, cytotoxicity and oxidative stress.