

# Characterization of an *in vitro* Whole Cigarette Smoke Exposure System (VITROCELL® 24/48)

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## Introduction

There has been rapid progress in approaches to assess the effects of cigarette whole smoke *in vitro*, enabling the exposure of cells at the air-liquid-interface (ALI) rather than in submerged condition applying smoke fractions (GVP/TPM) separately collected in a non-representative media. Only a few exposure systems are available to date allowing ALI exposure of cells in culture and one of the most advanced is the VITROCELL® system (VITROCELL Systems GmbH, Germany). To better understand its performance and to optimize the experimental conditions when exposing cells at the ALI, we recently characterized a VITROCELL® system connected to 30-port carousel smoking machine. The system (VITROCELL® 24/48) allows a simultaneous exposure of 48 cell culture inserts with either undiluted smoke or up to a maximum of 7 different smoke dilutions, with humidified air using flow rates of 0.1 – 3.0 L/min and exposing 6 inserts per dilution. These flow rates translate into smoke dilution ratios of 1:1.57 – 1:18.14 (smoke : air v/v) or 63% - 5.6% of whole smoke, respectively.

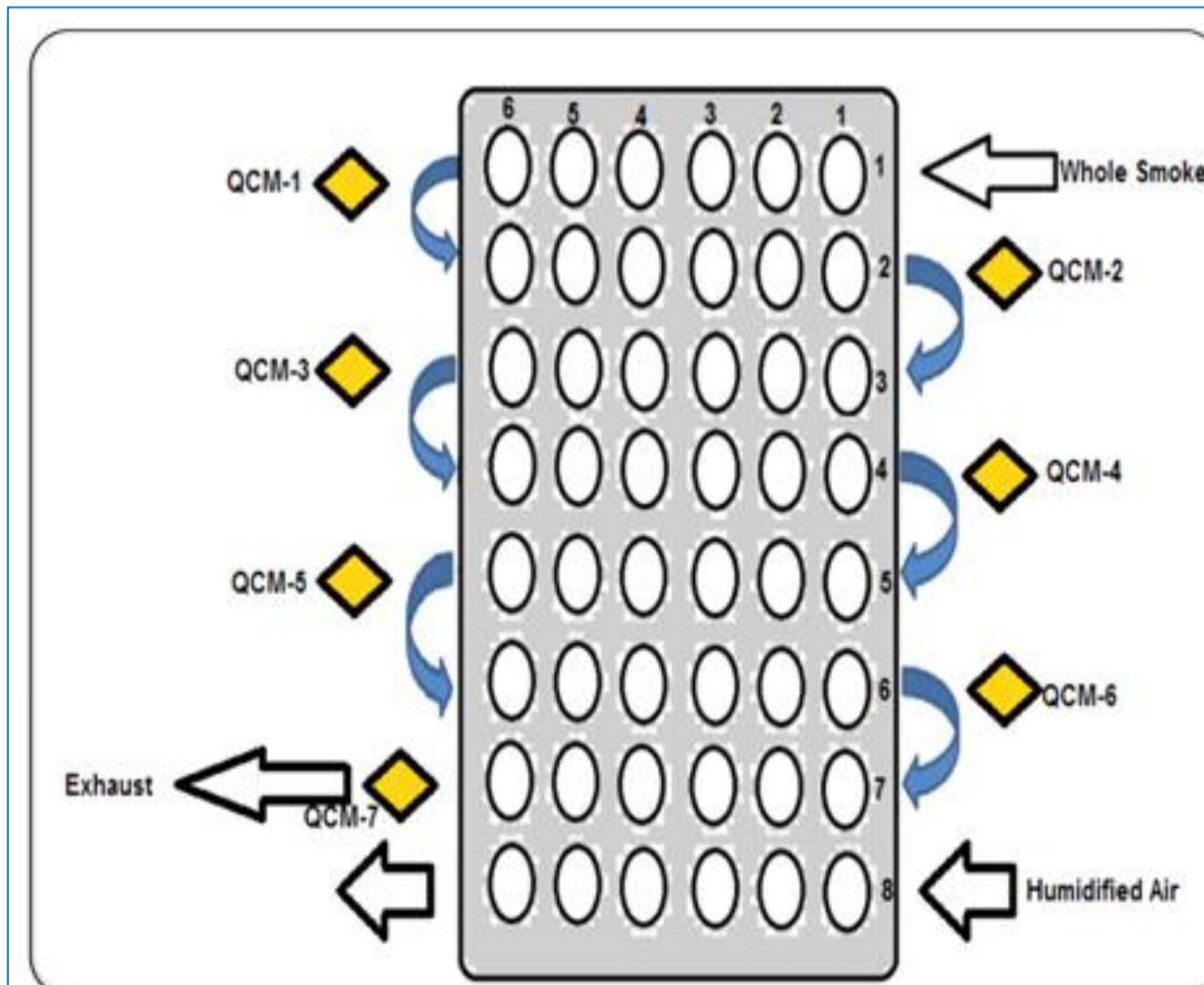
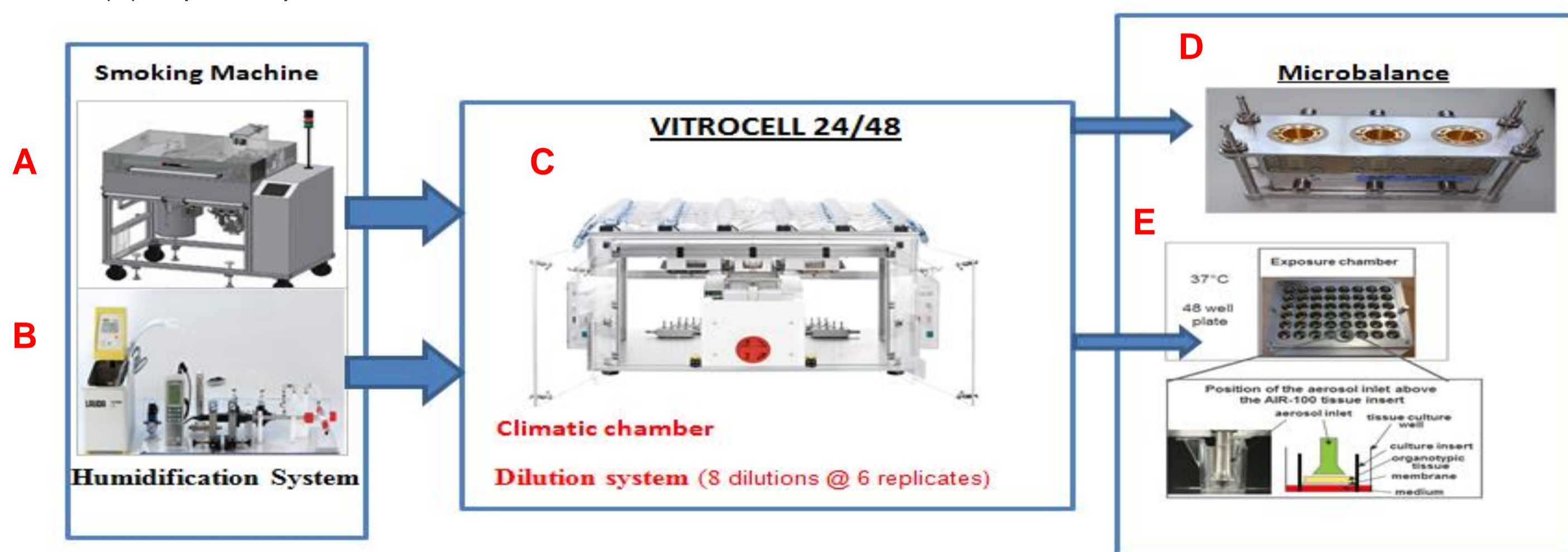
## Materials and Methods

**Determination of whole smoke deposition (WST-1 assay):** The well-to-well variability with same dilution (0.5L/min) and dose-dependent exposure with whole smoke was measured with WST-1 (an oxidation sensitive tetrazolium reagent). 1 mL of WST-1 was mixed with 4 mL Dulbecco's Modified Eagle's Medium (DMEM) accompanied with 0.1% Gentamycin. 75 µL of this mixture was added on cell culture inserts and exposed to the smoke of five Reference Cigarettes 3R4F. After exposure to whole smoke 50 µL WST-1 mixture was transferred into a 96-well plate and absorbance was measured at 430nm.

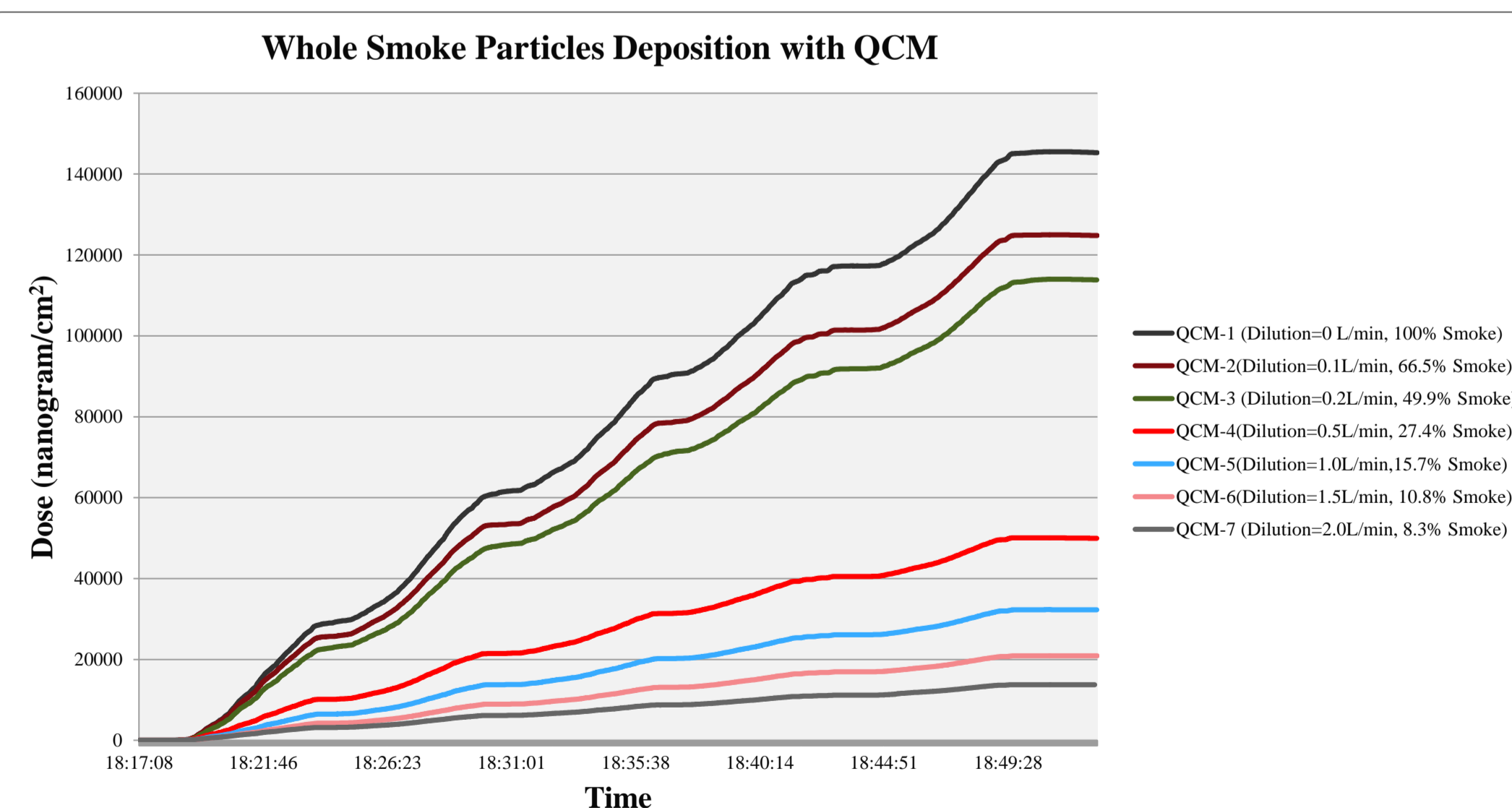
**Smoke generation:** 3R4F Reference Cigarettes were conditioned and smoked according to ISO conditions (1 puff/min of 35 mL volume and 2 second duration) on a 30-port carousel smoking machine. Whole cigarette smoke (8 puffs/cigarette) was used to expose the cells at the ALI for 8 minutes.

**Resazurin assay:** Following a 22 h post-exposure period upon exposure of BEAS-2B cells to different dilutions WS 150 µL resazurin solution was added per well and incubated for 2 additional hours at 37 °C, 5 % CO<sub>2</sub>, 95 % rel. humidity. At the end of the incubation time 100 µL/well were taken and fluorescence was measured for each well using 530-560nm excitation and 590nm emission wavelength.

**Figure 1: Schematic View of the Whole Smoke Exposure System (VITROCELL® 24/48):** (A) 30-port carousel smoking machine, (B) Humidification system for the humidification of dilution and the control air, (C) Climatic chamber to ensure constant temperature during the exposure, (D) VITROCELL® Quartz Crystal Microbalances (QCM) inaugurated with each dilution row for the dose-response measurement in the Climatic Chamber, (E) Exposure plate.



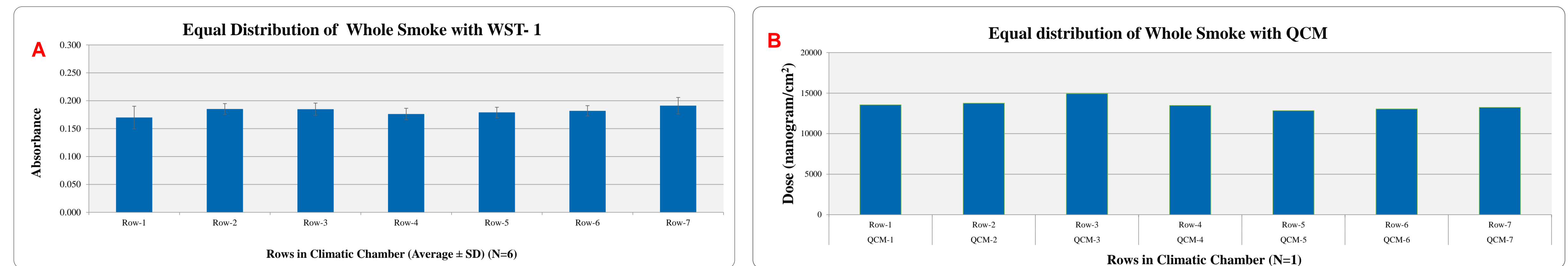
**Figure 2: Layout of the Exposure Plate with QCMs:** Each exposure row is connected to one VITROCELL® QCM to determine particles deposition for each dilution.



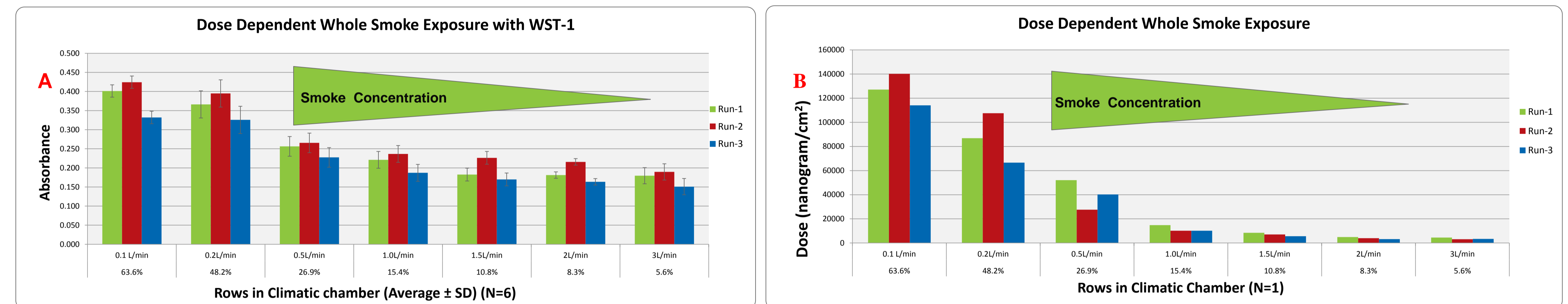
**Figure 3: Online Deposition of Whole Smoke Particles on the QCM:** Ten 3R4F Reference Cigarettes were smoked in 30 minutes duration with different dilutions of whole smoke and online particles deposition were measured with 7 QCMs.

## Results

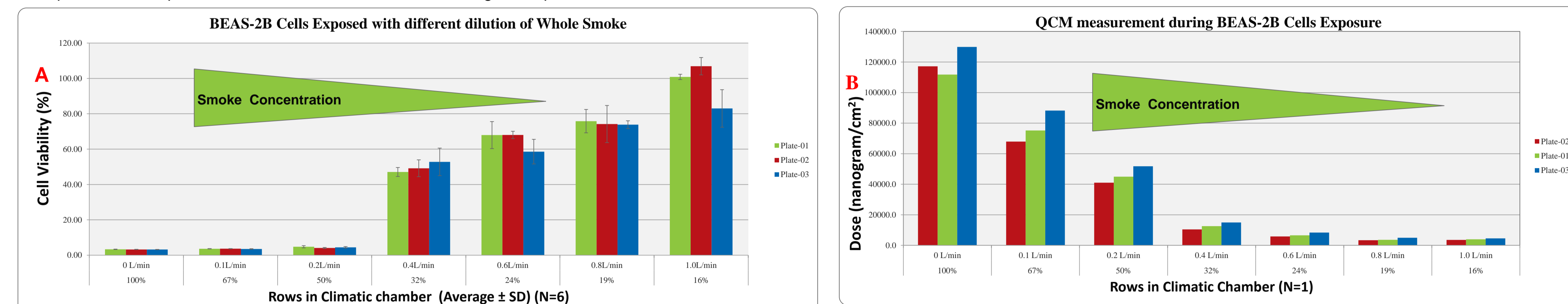
**Figure 4: Equal Distribution (Well-to-Well Variability) of Whole Smoke.** (A) Graph represents 7 rows in the climatic chamber with 6 inserts per row. Exposure experiments were repeated in triplicate. (B) Graph shows the deposition of the particles measured online during the exposure with fixed dilution of whole smoke (0.5L/min).



**Figure 5: Assessment of Dose Dependent (Multi-dilution) of Whole Smoke Exposure:** (A) Graph shows dose-dependent smoke exposure of the inserts with WST-1. Data were acquired in triplicate for all dilution levels. (B) Graph shows the deposition of the particles measured online during the exposure using different dilution flow rates of 0.1 – 3.0 L/min. QCM data also shows dose-dependent particles depositions.



**Figure 6: Whole Smoke Exposure using BEAS-2B cells :** (A) Graph shows cells viability of BEAS-2B cells following a 24 h post exposure. Data were acquired in triplicate for all dilution levels. (B) Graph shows the deposition of the particles measured online with QCM during the exposure of the BEAS-2B cells with undiluted and different dilution of whole smoke.



## Conclusions

- We successfully characterized the VITROCELL® 24/48 system to assess the effect of whole cigarette smoke on cells following exposure conditions more closely related to those occurring in human airways.
- Study results demonstrated an equal aerosol distribution of WS across all 42 inserts and the utility of VITROCELL® Crystal Quartz Microbalances (QCM) for determining the online deposition of smoke particles.
- The RSD (Relative standard deviation) for the well-to-well variability determined in the VITROCELL module after whole smoke exposure was 7% at a fixed smoke dilution flow of 0.5 L/min.
- Using different dilution flow rates of 0.1 – 3.0 L/min, a dose-dependent smoke exposure of the inserts could be demonstrated, determined by particle deposition on the QCM. In addition, we applied a colorimetric readout to confirm the dose-dependent exposure, which was achieved using an oxidation sensitive reagent (tetrazolium) added to empty cell culture inserts. Relative standard deviation (RSD) varies between different dilutions and it ranges from 10.8% to 17.8%.
- Cellular cytotoxicity of undiluted whole smoke as well as diluted whole smoke (dilution flow rates between 0 – 1.0 L/min) was determined by a Resazurin assay using lung epithelial BEAS-2B cells, grown in the inserts. A dose-dependent and reproducible decrease in cellular viability was identified.

