VITROCELL® Cloud Alpha

A Family of Exposure Systems Suitable for Nebulized Solutions and Suspensions

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Optimal Solution for Everyday Experiments at the Air/Liquid Interface

The VITROCELL® Cloud Alpha series is our newest innovation and presents a great leap forward in automated exposure of cell cultures. It combines reliable exposure of cell cultures from the respiratory tract with ease of use. The series comprises the Cloud Alpha 6, -12, -96 and Cloud Alpha MAX – they represent an optimal solution for everyday experiments at the Air/Liquid Interface using 6-well, 12-well, 24-well or 96-sized inserts.
We have run extensive tests to ensure the Cloud Alpha series devices match our standards in terms of the established characteristics for Cloud principle exposure devices: Deposition Efficiency, Spatial Deposition and Repeatability.

Fig. 1: VITROCELL® Cloud Alpha Family overwiew. Cloud Alpha 6, -12, -96+, -96 and Cloud Alpha MAX, left to right.

Methods

To determine the performance of the devices, Fluorescein-Sodium was nebulized with standard Aeroneb vibrating mesh nebulizers, imitating the procedure of a regular cell culture experiment and following the well described Cloud principle of operation.[1] Previously reported optimal nebulization volumes (see tab. 1) were used. The Fluorescein was trapped in PBS using stainless steel inserts. The concentration of deposited fluorescein was then determined via spectrofluorimetry. This method has been described in detail before.[1][2]
The characteristic values may the be calculated via:

$$(1)\quad DepositionEfficiency = \frac{C_{fluo.Insert} * V_{catch.Insert}}{C_{fluo.Nebulized} * V_{Nebulized} * \frac{A_{Insert}}{A_{Chamber}}}$$

$$(2)\quad SpatialDeposition = \sqrt{\frac{\sum_{i=1}^{n}(c_{fluo.Insert_i} - \mu_i)^2}{n}} * \frac{1}{\mu_i}$$

The shown efficiencies are values averaged over all inserts and over several experiments (minimum 3, maximum 9). The variance between this averaged efficiency per run has then been used as a measure for repeatability. To adress the different ratios between exposed area and cell culture area per device, we propose the measure Insert Deposition Efficiency:

$$(3)\quad InsertDepositionEfficiency = DepositionEffciency * \frac{A_{Culture}}{A_{Chamber}}$$

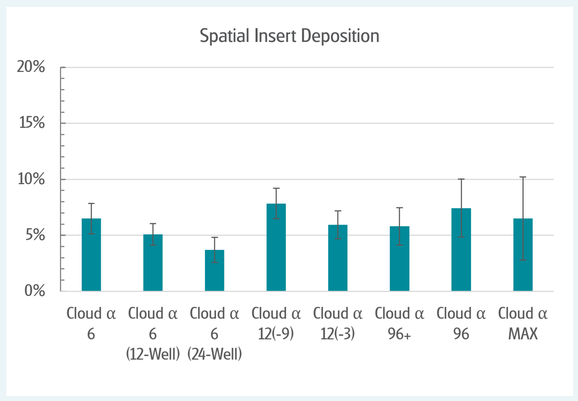

Fig. 2: Insert-to-insert variabilty per device type (n left to right: 4, 4, 4, 9, 7, 7, 6)

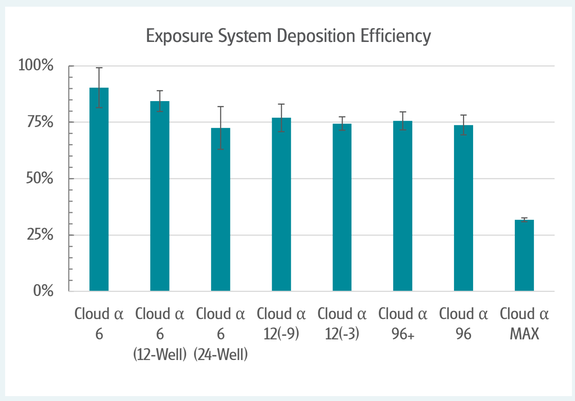

Fig. 3: Efficiency (overall and culture related) in available devices

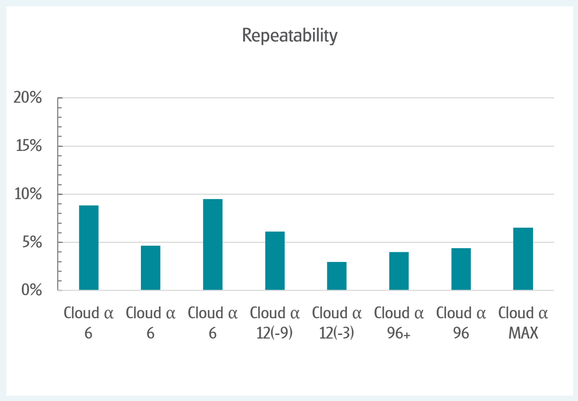

Fig. 4: Experiment-to-experiment variability in available devices

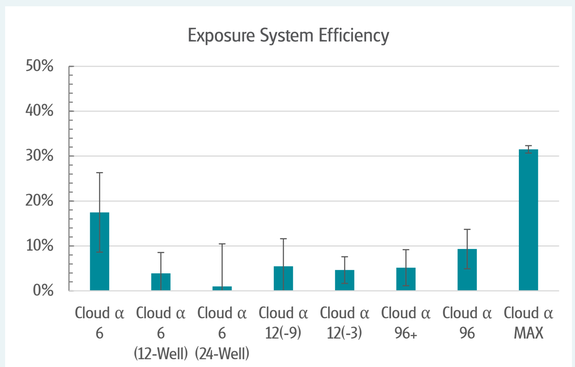

Fig. 5: Cell culture efficiency

		Alpha 6			Alpha 12		Alpha 96+	Alpha 96	Alpha MAX
		6-well	12-well	24-well	12-9	12-3			
Deposition Efficiency		90%	84%	72%	77%	74%	76%	74%	32%
Exposure area / (cm^2)		145	145	145	141,5	54	200,75	108,75	4,71
Culture growth area / (cm^2)		28,0	6,7	2,0	10,1	3,4	13,7	13,7	4,7
Insert Deposition Efficiency		17%	4%	1%	5%	5%	5%	9%	32%
Optimal deposition for standard nebulization of 1% solution / (µg/cm^2)		13,8	13,8	13,8	14,1	14,8	14,9	18,4	42,5
Nebulization volume / (µL)	min	50			50	30	300	30	10
	standard	200			200	80	300	200	20
	max	400			400	130	300	300	40

Tab. 1: Areas, Volumes and Efficiencies of the VITROCELL® Cloud Alpha devices

Features:

- Cloud dynamics by substance-safe vibrating mesh nebulizers
- Programmable, automated exposures
- Very low insert-to-insert variabilty/high reproducibility
- Air Liquid Interface (ALI) cell culture exposure
- Direct guidance of test substance to the cells
- No external airflow required

Fig. 6: Touchscreen control of VITROCELL® Cloud Alpha devices

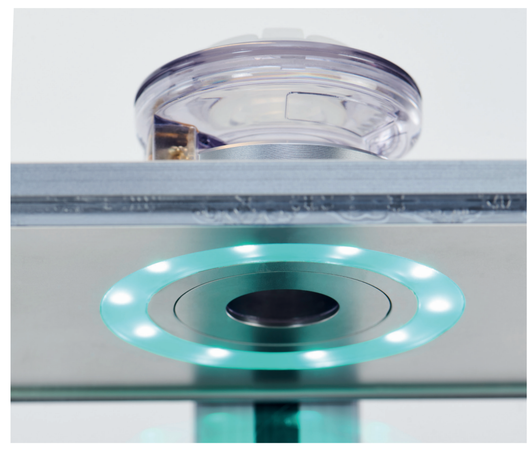

Fig. 7: Vibrating mesh nebulizers for aerosol generation

[1] Lenz et al, *A dose-controlled system for air-liquid interface cell exposure and application to zinc oxide nanoparticles*, Particle and Fibre Toxicology, page 3 ff., 2009 (DOI: 10.1186/1743-8977-6-32)
[2] Ding et al, *Quartz crystal microbalances (QCM) are suitable for real-time dosimetry in nanotoxicological studies using VITROCELL® Cloud cell exposure systems*, Particle and Fibre Toxicology, page 6 f., 2020 (DOI: 10.1186/12989-020-00376)