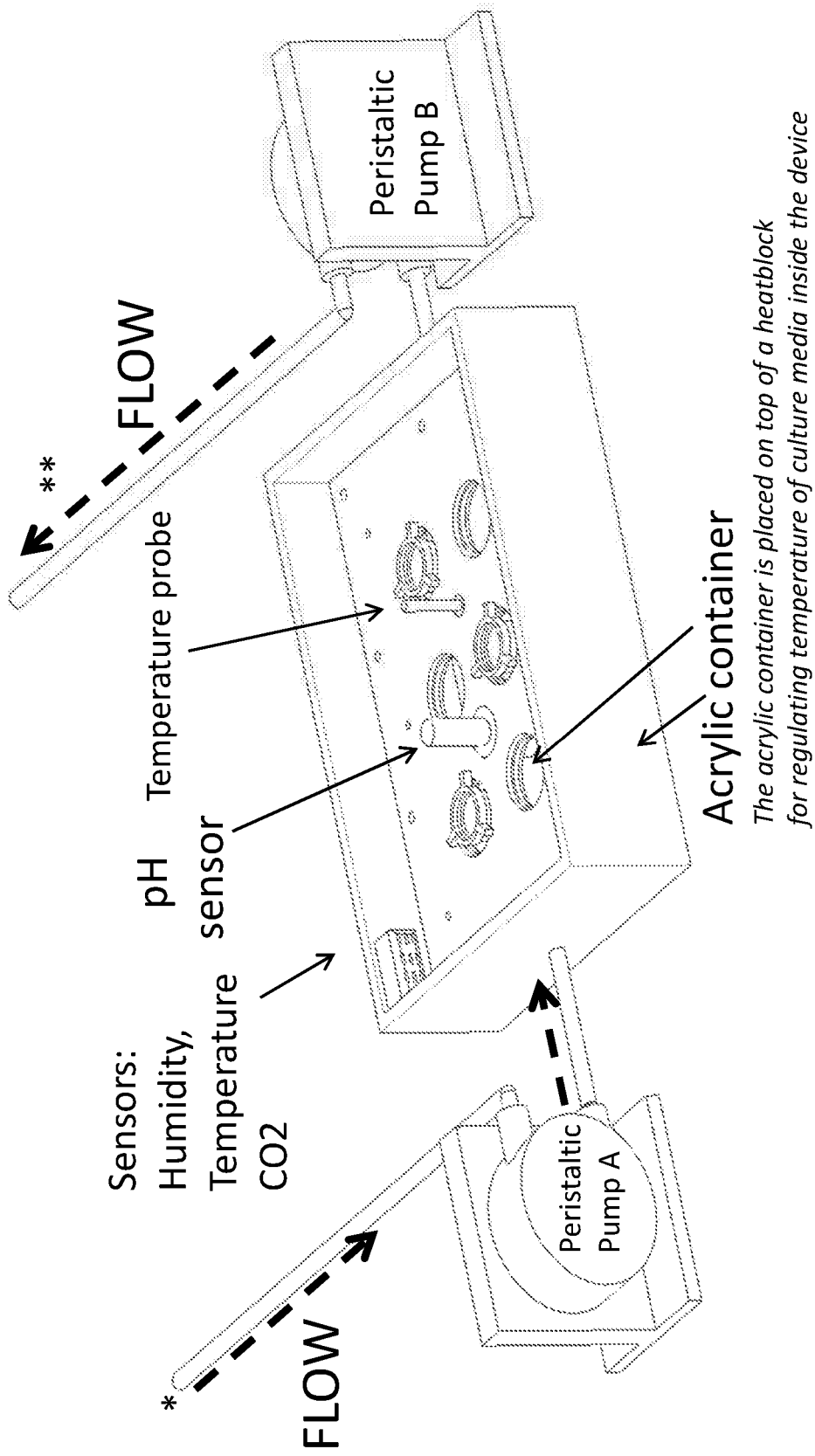
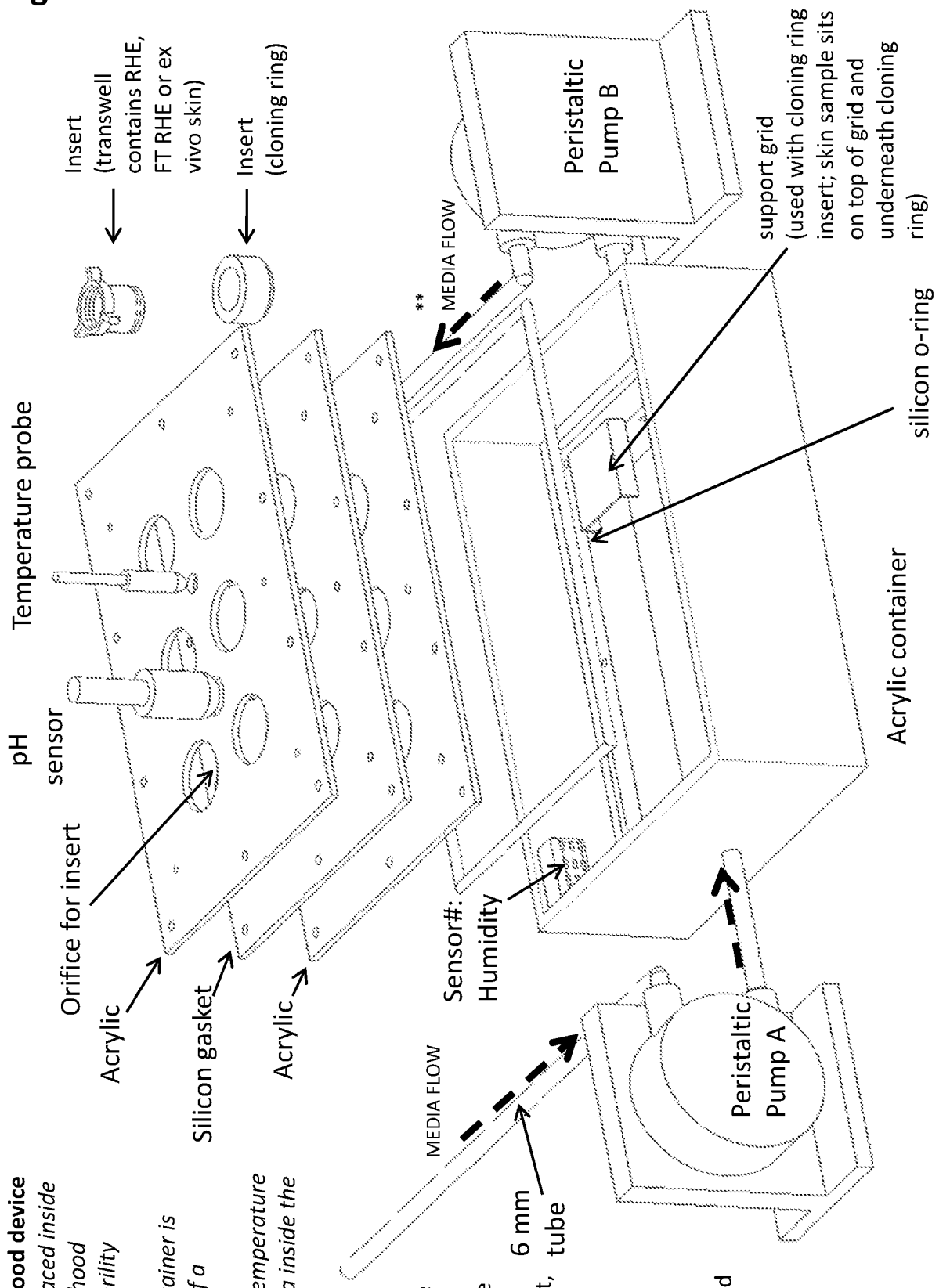

Peristaltic Pump B
FLOW
**
Temperature probe
pH sensor
Sensors: Humidity, Temperature CO2
Acrylic container
The acrylic container is placed on top of a heatblock for regulating temperature of culture media inside the device
Peristaltic Pump A
FLOW
*
* Inlet linked to source bottle of CO2 buffered culture media
** Outlet, returns culture media to CO2 buffered source

Figure 1A

Figure 1b

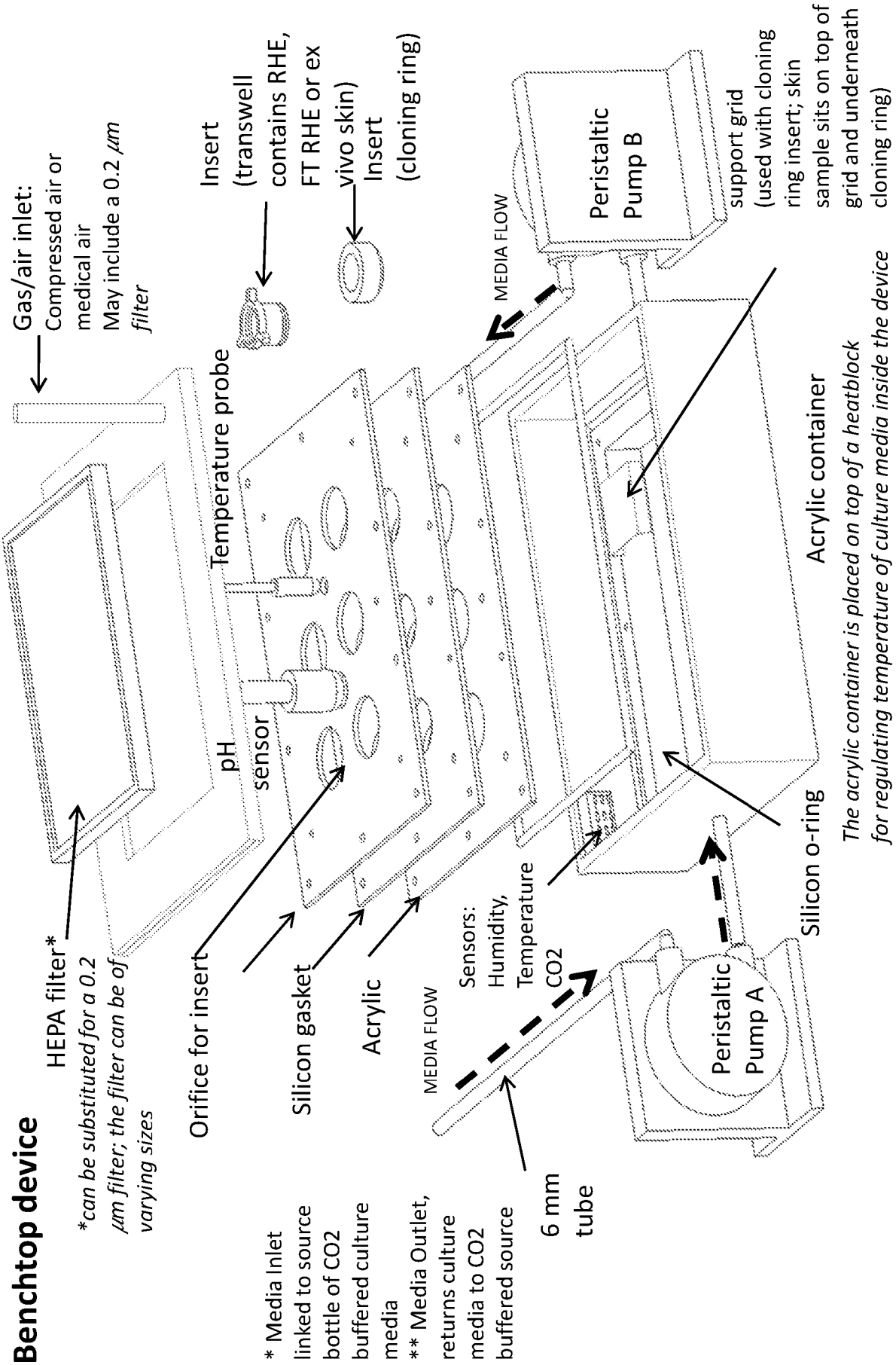


Laminar flow hood device
The device is placed inside a laminar flow hood to maintain sterility

The acrylic container is placed on top of a heatblock for regulating temperature of culture media inside the device

* Media Inlet linked to source bottle of CO_2 buffered culture media
** Media Outlet, returns culture media to CO_2 buffered source
# An additional CO_2, temperature and humidity monitor is placed next to the device to continually assess atmosphere

Figure 1C


Benchtop device
Gas/air inlet:
Compressed air or medical air
May include a 0.2 µm filter
HEPA filter*
*can be substituted for a 0.2 µm filter; the filter can be of varying sizes
Insert (transwell contains RHE, FT RHE or ex vivo skin)
Insert (cloning ring)
Temperature probe
pH sensor
MEDIA FLOW
Peristaltic Pump B
support grid (used with cloning ring insert; skin sample sits on top of grid and underneath cloning ring)
Acrylic container
The acrylic container is placed on top of a heatblock for regulating temperature of culture media inside the device
Orifice for insert
Silicon gasket
Acrylic
MEDIA FLOW
Sensors: Humidity, Temperature CO2
Peristaltic Pump A
Silicon o-ring
* Media Inlet linked to source bottle of CO2 buffered culture media
** Media Outlet, returns culture media to CO2 buffered source
6 mm tube

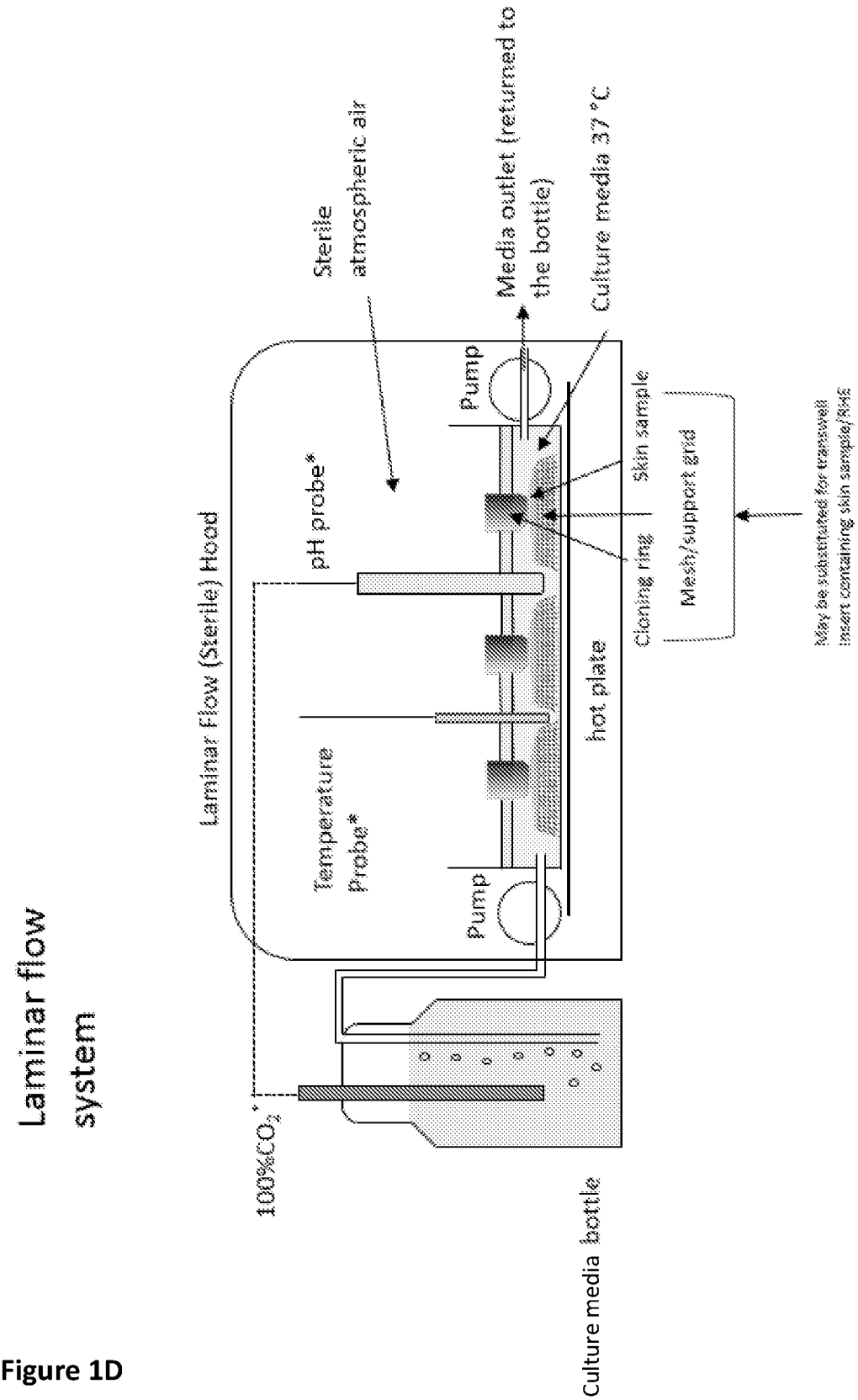

Laminar flow system
Laminar Flow (Sterile) Hood
Sterile atmospheric air
Media outlet (returned to the bottle)
Culture media 37 °C
Pump
pH probe*
Skin sample
Cloning ring
Mesh/support grid
Temperature Probe*
hot plate
Pump
100%CO_2*
Culture media bottle
May be substituted for transwell insert containing skin sample/RHE

Figure 1D

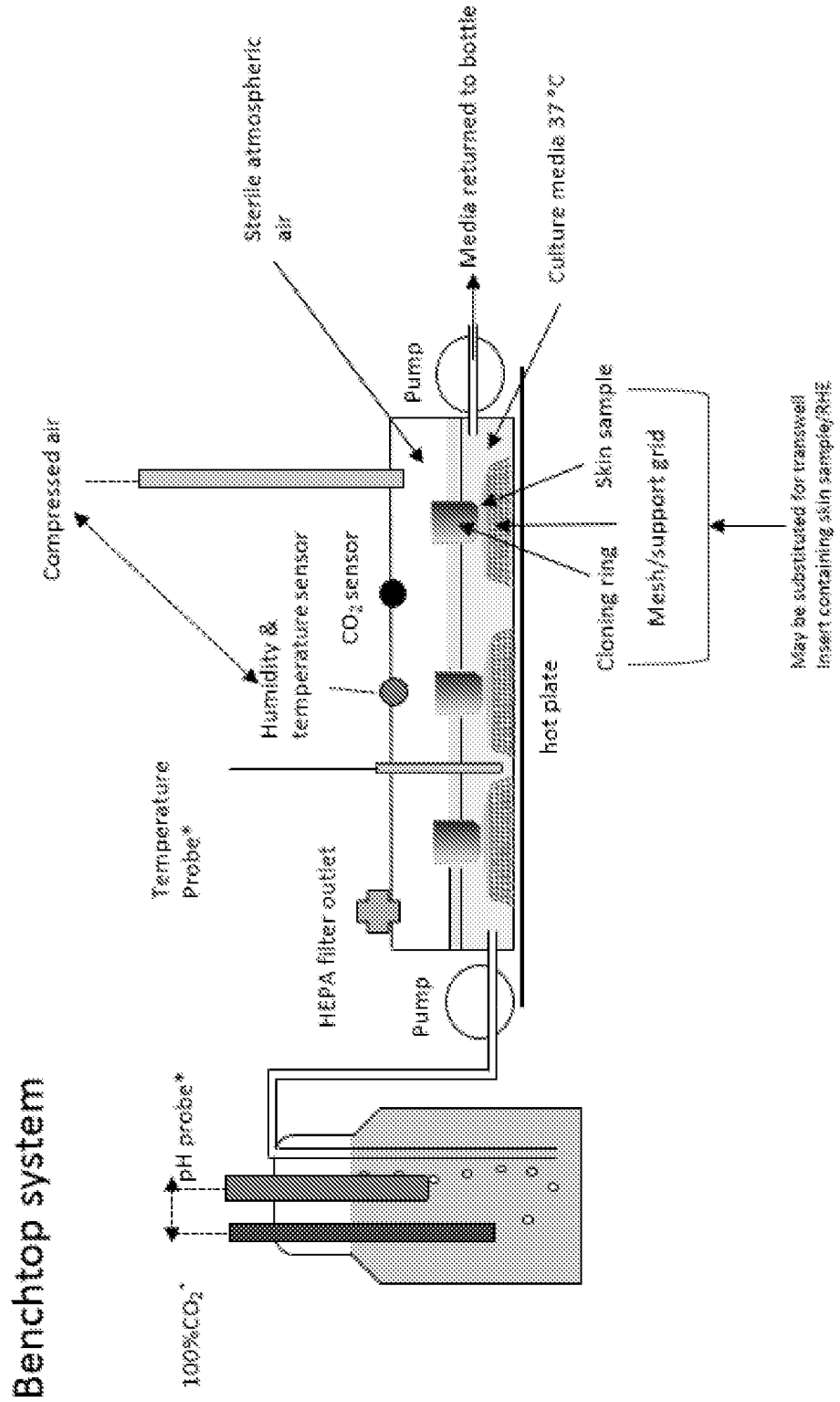
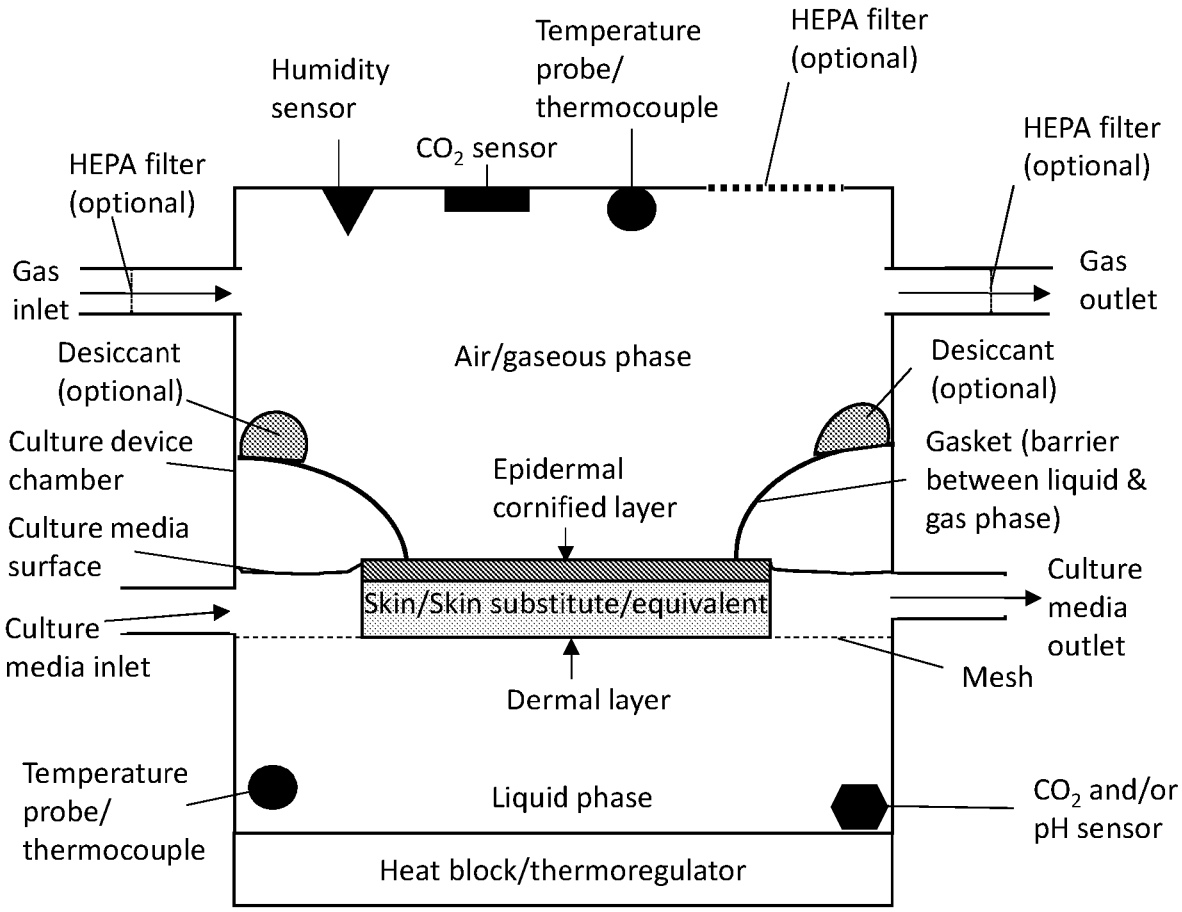

Benchtop system
100%CO_2
pH probe*
HEPA filter outlet
Temperature Probe*
Humidity & temperature sensor
CO_2 sensor
Compressed air
Sterile atmospheric air
Pump
Media returned to bottle
Culture media 37°C
Skin sample
Cloning ring
Mesh/support grid
hot plate
May be substituted for transwell insert containing skin sample/RHE

Figure 1E

Figure 1F


Humidity sensor
Temperature probe/ thermocouple
HEPA filter (optional)
CO_2 sensor
HEPA filter (optional)
HEPA filter (optional)
Gas inlet
Gas outlet
Air/gaseous phase
Desiccant (optional)
Desiccant (optional)
Culture device chamber
Gasket (barrier between liquid & gas phase)
Epidermal cornified layer
Culture media surface
Skin/Skin substitute/equivalent
Culture media outlet
Culture media inlet
Mesh
Dermal layer
Temperature probe/ thermocouple
Liquid phase
CO_2 and/or pH sensor
Heat block/thermoregulator

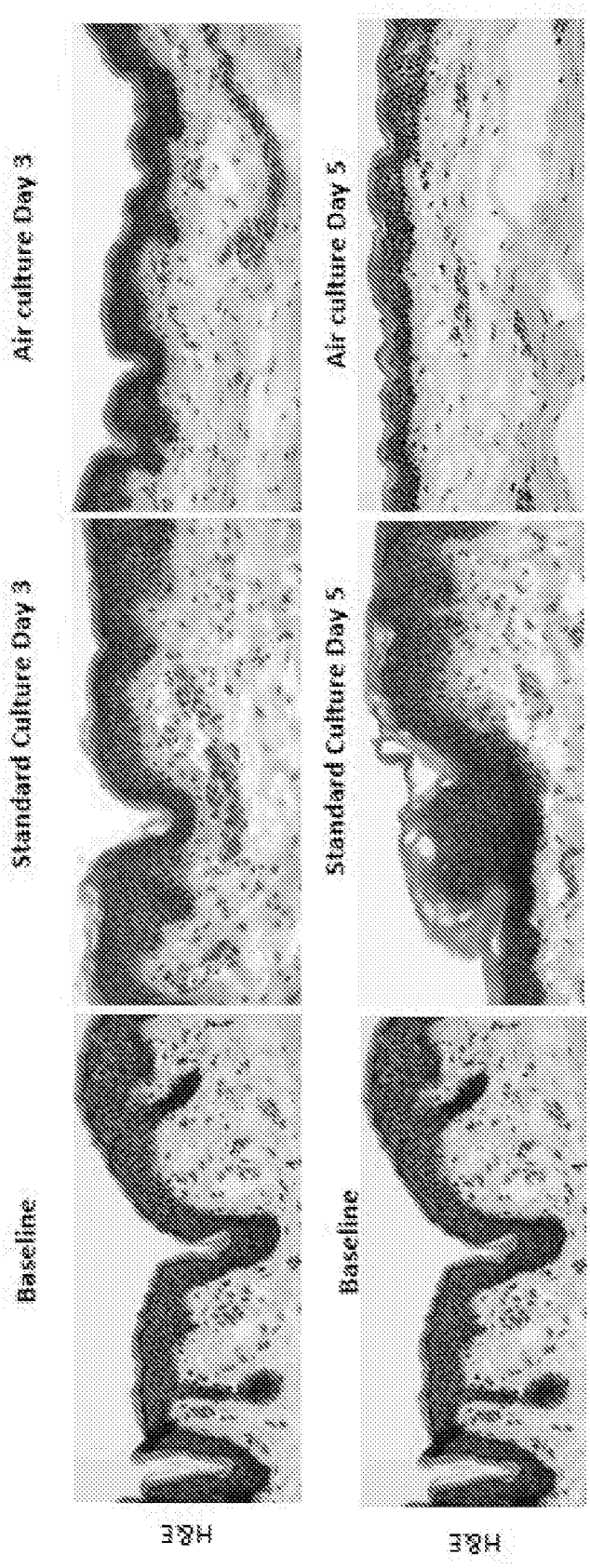

Air culture Day 3
Air culture Day 5
Standard Culture Day 3
Standard Culture Day 5
Baseline
Baseline
HBE
HBE

Figure 2A

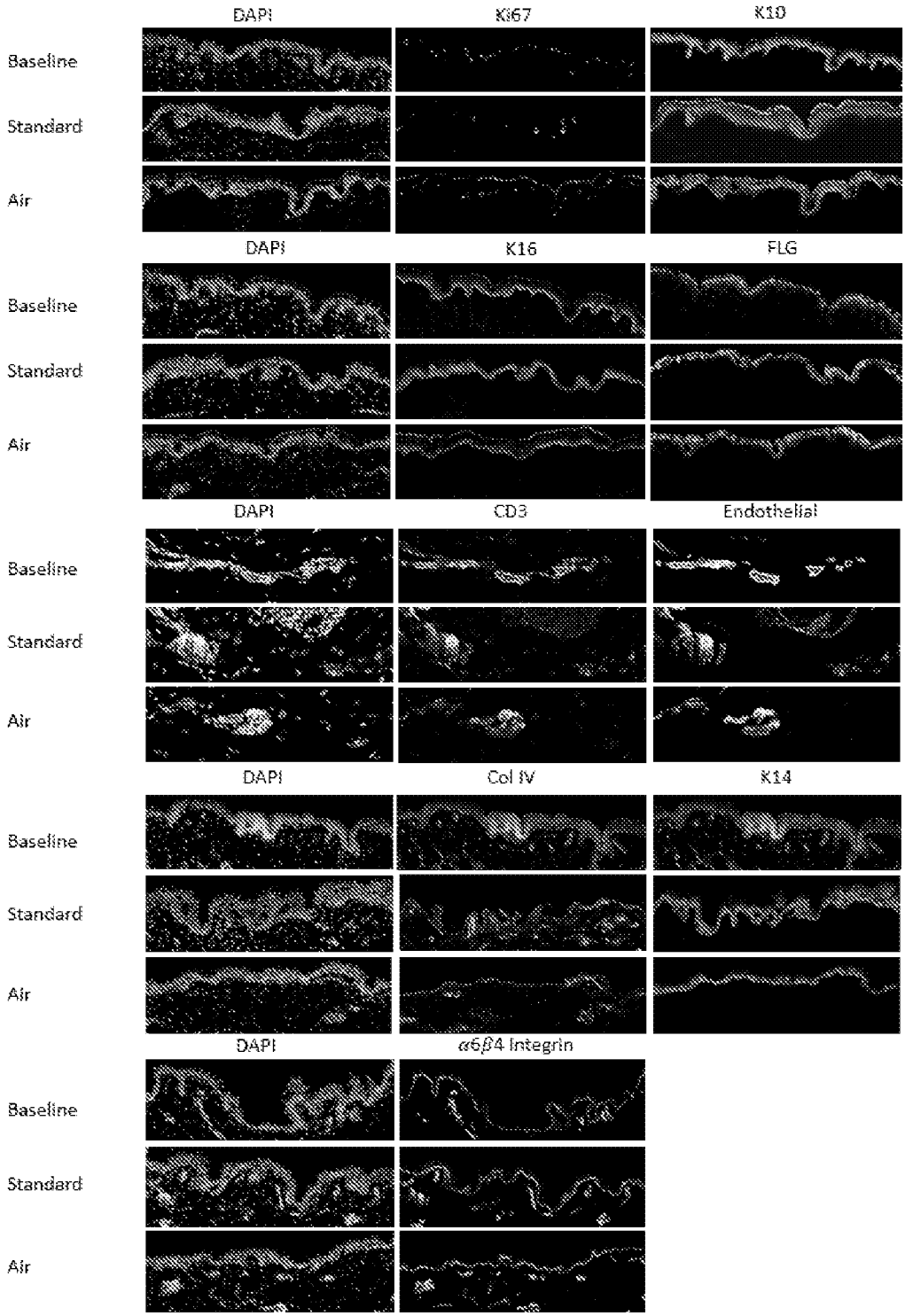

DAPI
Ki67
K10
Baseline
Standard
Air
DAPI
K16
FLG
Baseline
Standard
Air
DAPI
CD3
Endothelial
Baseline
Standard
Air
DAPI
Col IV
K14
Baseline
Standard
Air
DAPI
α6β4 Integrin
Baseline
Standard
Air

Figure 2B

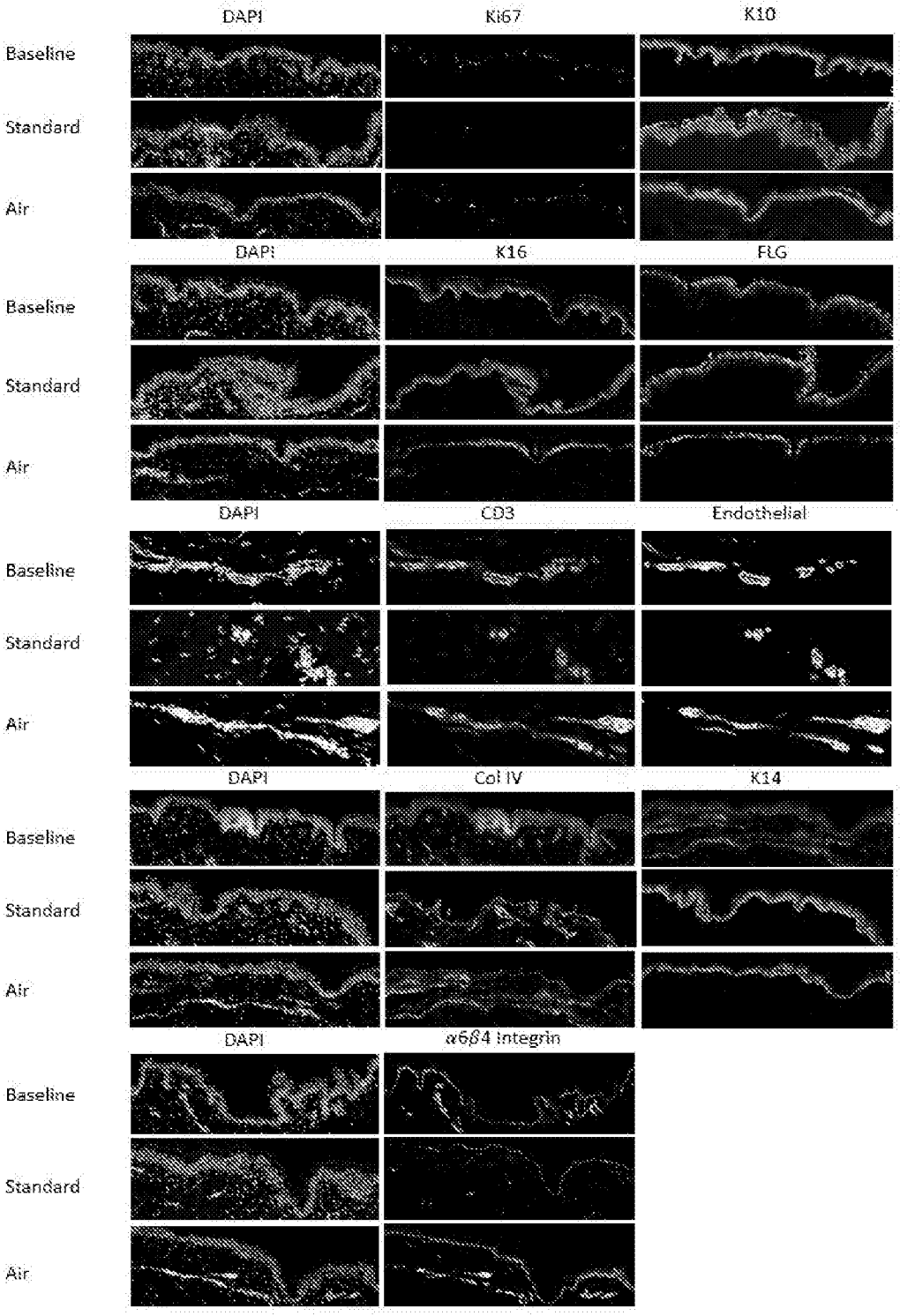

DAPI
Ki67
K10
Baseline
Standard
Air
DAPI
K16
FLG
Baseline
Standard
Air
DAPI
CD3
Endothelial
Baseline
Standard
Air
DAPI
Col IV
K14
Baseline
Standard
Air
DAPI
α6β4 Integrin
Baseline
Standard
Air

Figure 2C

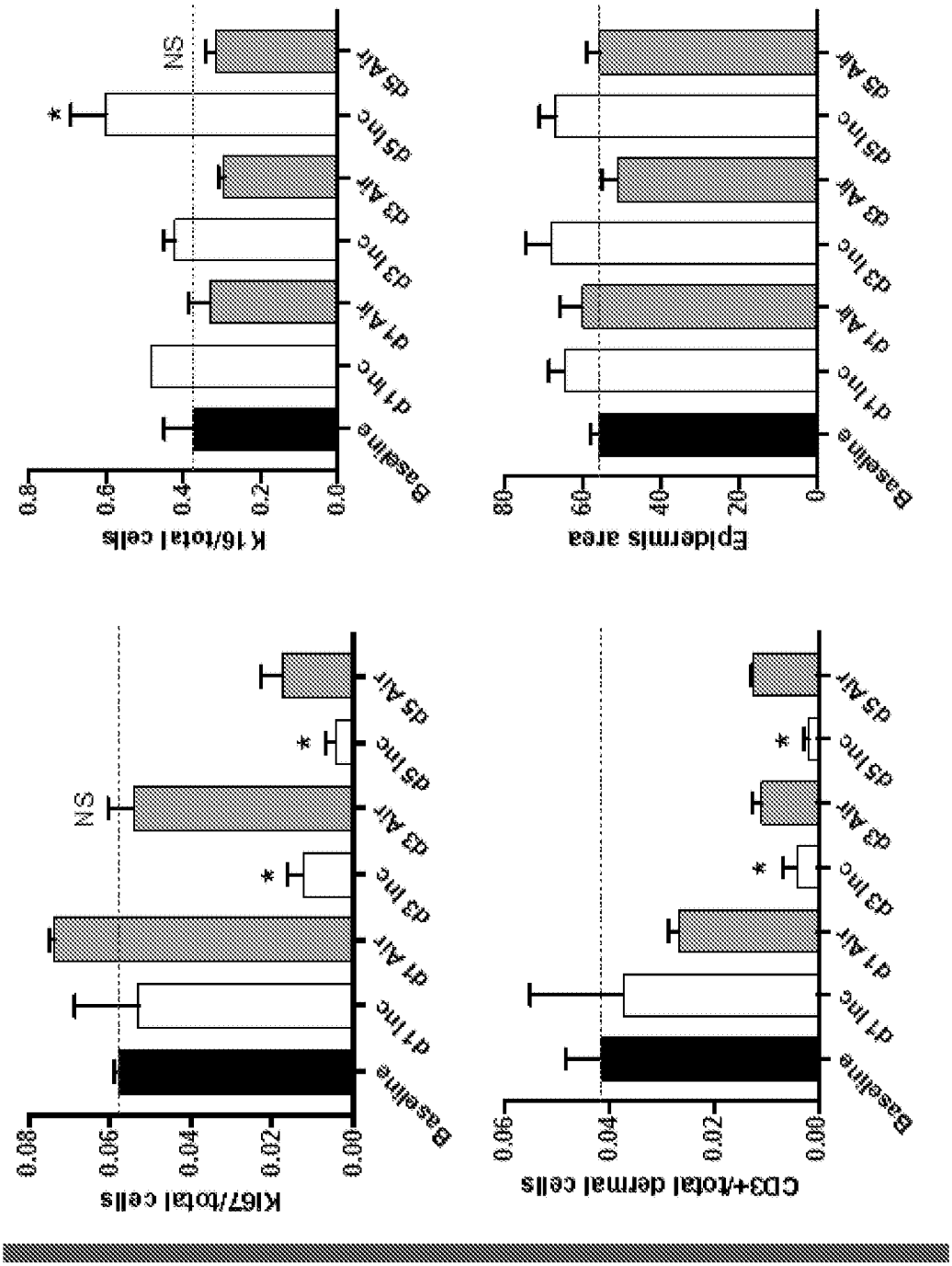

Baseline
d1 Inc
d1 Air
d3 Inc
d3 Air
d5 Inc
d5 Air
CD3+/total dermal cells
0.00
0.02
0.04
0.06
*
*
Epidermis area
0
20
40
60
80
K16/total cells
0.0
0.2
0.4
0.6
0.8
NS
*
K167/total cells
0.00
0.02
0.04
0.06
0.08
NS
*
*

Figure 3

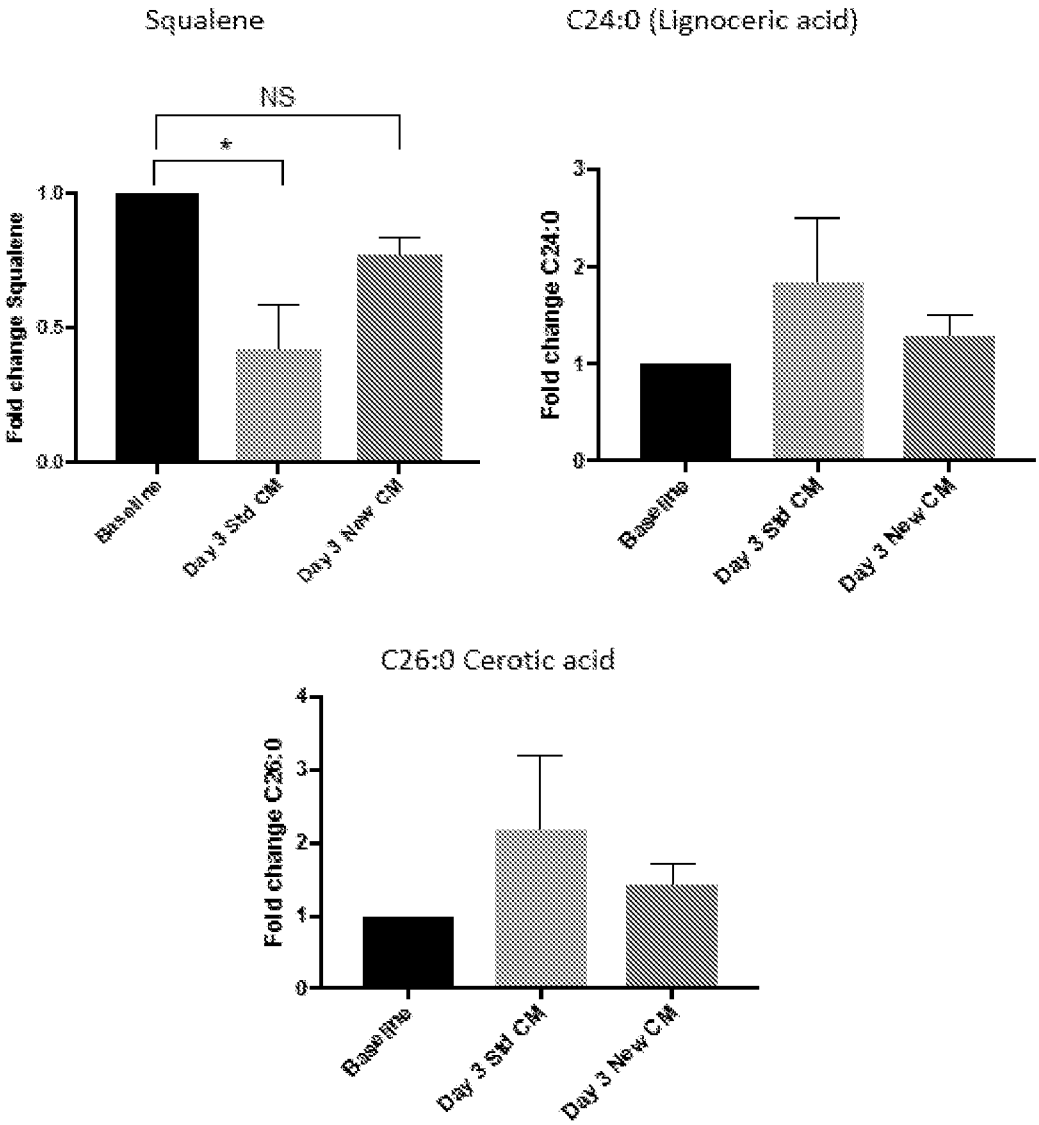

Squalene
NS
*
Fold change Squalene
1.0
0.5
0.0
Baseline
Day 3 Std CM
Day 3 New CM
C24:0 (Lignoceric acid)
Fold change C24:0
3
2
1
0
Baseline
Day 3 Std CM
Day 3 New CM
C26:0 Cerotic acid
Fold change C26:0
4
3
2
1
0
Baseline
Day 3 Std CM
Day 3 New CM

Figure 4

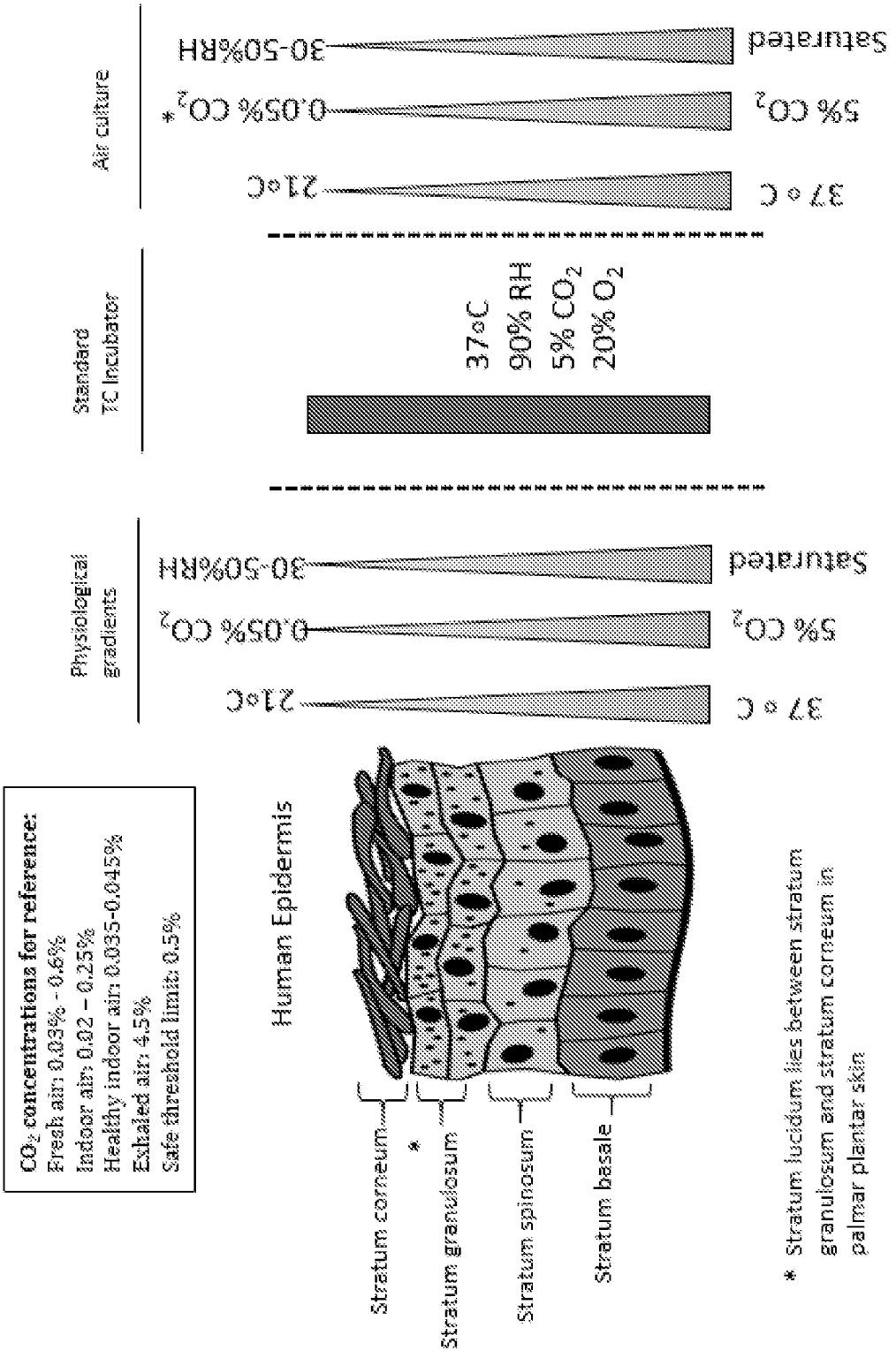

Physiological gradients
37 ◦ C
21◦C
5% CO_2
0.05% CO_2
Saturated
30-50%RH
Standard TC Incubator
37◦C
90% RH
5% CO_2
20% O_2
Air culture
37 ◦ C
21◦C
5% CO_2
0.05% CO_2*
Saturated
30-50%RH
CO_2 concentrations for reference:
Fresh air: 0.03% - 0.6%
Indoor air: 0.02 – 0.25%
Healthy indoor air: 0.035-0.045%
Exhaled air: 4.5%
Safe threshold limit: 0.5%
Human Epidermis
Stratum corneum
*
Stratum granulosum
Stratum spinosum
Stratum basale
* Stratum lucidum lies between stratum granulosum and stratum corneum in palmar plantar skin

Figure 5

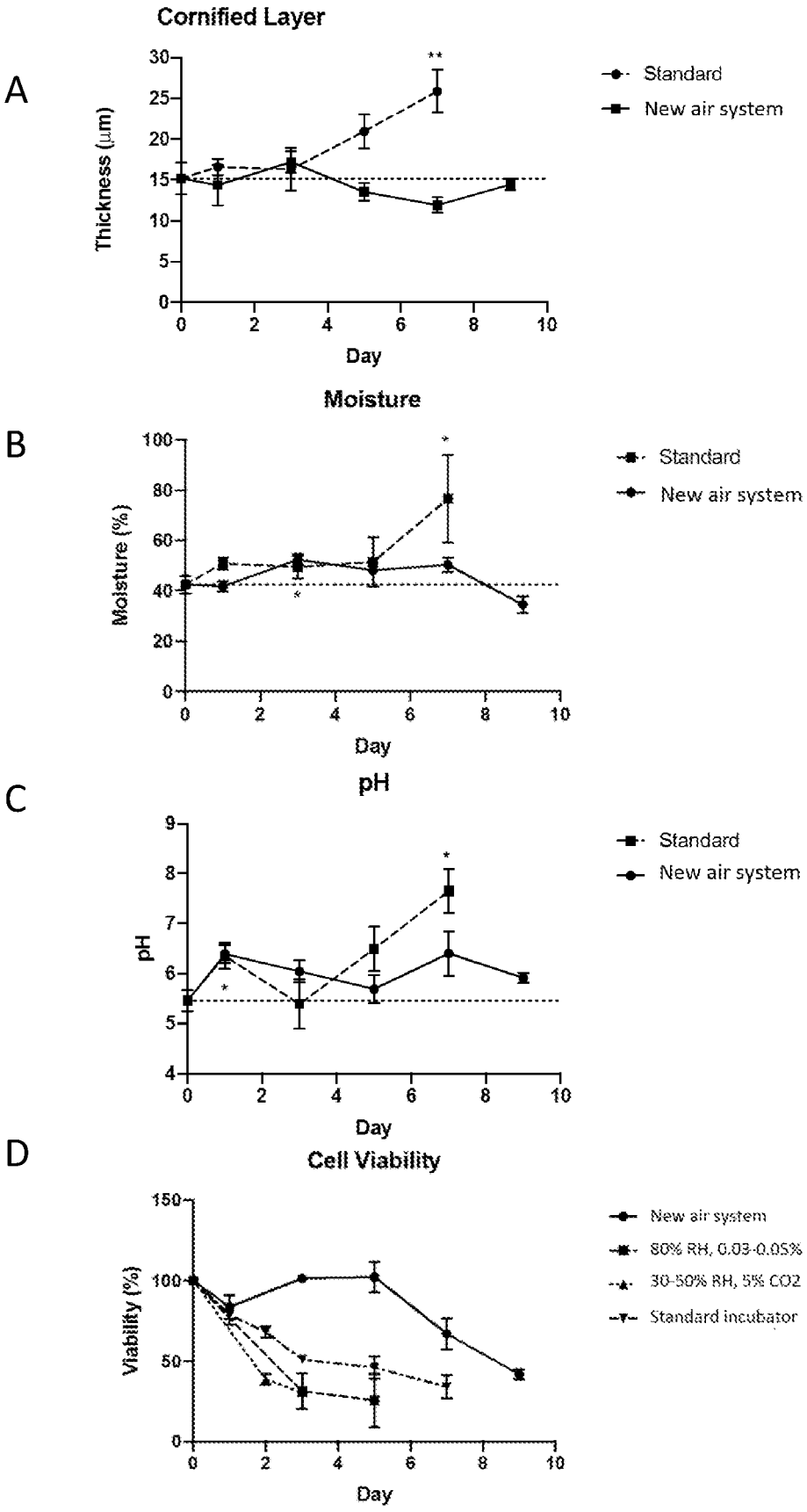

A
Cornified Layer
Thickness (µm)
Day
Standard
New air system
B
Moisture
Moisture (%)
Day
Standard
New air system
C
pH
pH
Day
Standard
New air system
D
Cell Viability
Viability (%)
Day
New air system
80% RH, 0.03-0.05% CO2
30-50% RH, 5% CO2
Standard incubator

Figure 6