Air-Liquid Interface (ALI) Guideline

Mar 2024

EG-182 Rev A

ALI in the Human Emulation System

Cell culture models under Air-Liquid Interface (ALI) allow for the study and understanding of biological interfaces between cells in airspace and the vascular networks within the human body. Some examples of ALI *in vivo* include lung (alveolus and airway), skin, upper respiratory tract, eye, tongue, and ear canal. Emulate's Human Emulation System enables ALI cultures to be established using the Chip-S1® Stretchable Chip or the Chip-A1™ Accessible Chip in Zoë® Culture Module.

Inducing ALI in the Chip-S1 and Chip-A1 involves a flush of air pressure through the Pod® Portable Module and chip channels using a flow rate of 1,000 µL/hr for 1 minute to push the residing media out of the channel and into the Pod outlet reservoir.

ALI Induction Steps

The following steps outline the general ALI induction workflow. This workflow may need to be modified according to specific protocols or as needed for organ-specific conditions.

1. Perform a complete aspiration of the Pod inlet and outlet reservoirs for the channel that will be placed under air (see Figure 1).

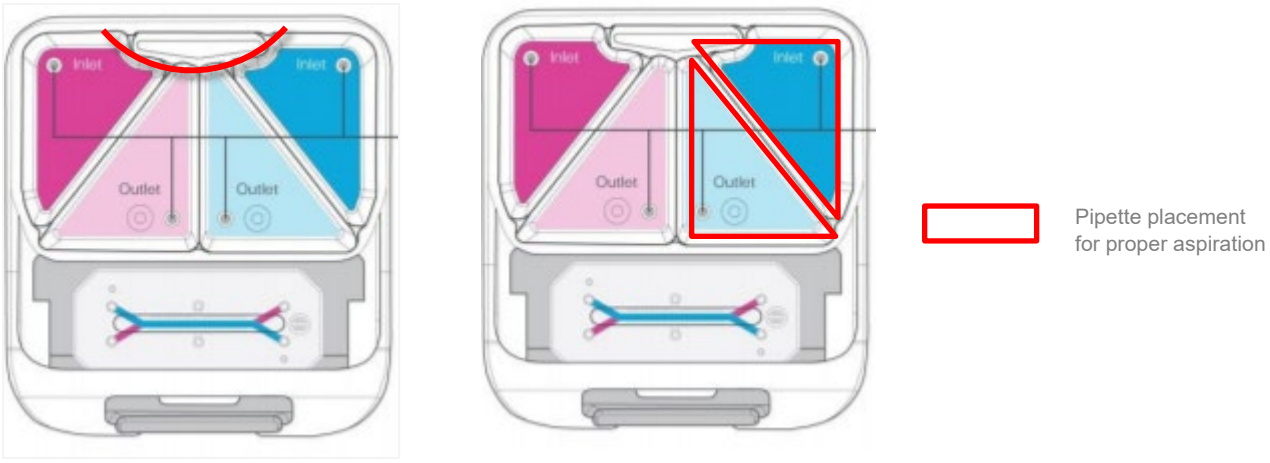

***Figure 1**. The images above demonstrate the proper position of the aspirating tip for either standard (left image) or "complete" (right image) aspiration. The figure on the right indicates specific placement of the aspirator tip to ensure complete aspiration of the top channel for ALI induction. Image depicts a Chip-S1 connected to the Pod, but the same techniques apply for Chip-A1 as well.*

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2. Place chips and Pods in Zoë and set the flow rate to 1,000 µL/hour in the air channel, and 0 µL/hour in the liquid channel. Flush the air channel by running flow on Zoë for 60 seconds.
3. Immediately remove Pods from Zoë and completely aspirate the Pod outlet reservoir corresponding to the air channel. This will prevent any outflow liquid from wicking back into the air channel.
4. Pipette 1x HBSS or PBS into the Pod inlet reservoir corresponding to the air channel first, then immediately pipette the same volume of 1x PBS or HBSS into the corresponding Pod outlet reservoir.
    i. The liquid covering the Pod reservoir Vias acts as a liquid lock in the air channel and prevents excess evaporation in the chip.
    ii. Equal media distribution in the inlet and outlet Pod reservoirs corresponding to the air channel is required to maintain static pressure.
    iii. The recommended volume for this step should be approximately 10% greater than the inlet reservoir volume corresponding to the liquid-filled channel. For example, if the inlet reservoir volume for the liquid-filled channel is 3.0 mL, then fill the inlet and outlet reservoirs for the air channel to 3.3 mL.
5. Switch to the ALI culture medium by aspirating the Pod inlet and outlet reservoirs corresponding to the liquid-filled channel following the standard aspiration technique (Figure 1), leaving a liquid layer above the Vias to prevent introduction of unwanted bubbles. Add the desired volume of warmed and gas-equilibrated ALI culture medium into the inlet reservoir corresponding to the liquid-filled channel.
6. Return Pods to Zoë and set the air channel flow rate to ‘Air’ or 0 µL/hour. Set the liquid channel flow rate according to the specific protocol requirements.

Key Factors and Best Practices

There are three key factors that should be taken into consideration when introducing ALI to Organ-Chip models in Zoë:

1. Reducing bubbles caused by serum in the media
2. Complete aspiration of the Pod reservoirs
3. Adherence to specific flush flow rate and duration*

* Note: Please ensure that the 1,000 µL/hr flow rate is run for exactly 1 minute. Anything longer could result in pod foaming and downstream damage to Zoë.

1. Serum Concentration of Media

Surfactants, including serum, in cell culture media can change its properties and may lead to foaming in the Pod outlet. The serum concentration in the media used should be taken into consideration when introducing ALI.

If the media composition in the ALI induction channel contains serum at a concentration of 2% or less, it is not expected to see significant foaming in the outlet reservoir. However, if the media in the channel has a serum concentration above 2%, it may be beneficial to first wash the channel with a reduced-serum or serum-free medium prior to inducing ALI. To do this, add 1 mL of reduced-serum or serum-free media to the Pod inlet reservoir for the channel of interest, and flush through the chip by flowing the channel of interest at 1,000 µL/hr for 1 minute before proceeding with the ALI induction.

2. Complete Aspiration of Pod Reservoirs

In the general chip maintenance workflow (media replenishment, compound dosing, effluent collection), aspiration is performed by carefully avoiding the Pod reservoir Vias, thus leaving a liquid layer over the Vias to prevent introduction of unwanted bubbles. However, when introducing air to the channel during ALI induction, a complete aspiration of the Pod reservoir is essential to ensure that the minimal volume of media is flushed through and out of the chip. This involves aspirating along the entire reservoir edges (see Figure 1), while still avoiding direct contact with the Vias.

Additionally, after the ALI induction step is completed, it is important to immediately use the same aspiration technique to remove all flushed media from the Pod outlet reservoir to prevent media backflow into the chip channel.

3. Time and Flow Rate

The flush steps should be timed and monitored to avoid flushing excess air pressure through the channel, which can be detrimental to sensitive cell types and create bubbles in the Pod outlet reservoir. With complete aspiration of the Pod reservoirs, the chip channels are cleared of media in under 1 minute when a flow rate of 1,000 µL/hour is used. This applies for both the Chip-S1 and Chip-A1. In general, ALI induction can be carried out in either channel by flushing air through a completely aspirated Pod reservoir at a flow rate of 1,000 µL/hour for 1 minute.

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Troubleshooting

<table>
<tr><th>If…</th><th>Then…</th></tr>
<tr><td>A bubble is observed in the liquid channel during ALI</td><td>Perform the following steps:
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Disconnect the chip from the Pod.</td></tr>
<tr><td>2</td><td>Manually flush the liquid channel of the chip with media.</td></tr>
<tr><td>3</td><td>Aspirate the liquid lock in the inlet and outlet corresponding to the air channel.</td></tr>
<tr><td>4</td><td>Re-Prime the Pod.</td></tr>
<tr><td>5</td><td>Reconnect the chip to the Pod.</td></tr>
<tr><td>6</td><td>Run a Regulate Cycle.</td></tr>
<tr><td>7</td><td>Re-introduce ALI.
Note: If using a Zoë-CM2, it is advisable to run Single-channel Regulate</td></tr>
</table></td></tr>
<tr><td>A bubble is observed in the air channel during ALI</td><td>Perform the following steps:
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Completely aspirate the liquid lock in the inlet reservoir corresponding to the air channel.</td></tr>
<tr><td>2</td><td>Perform a 1000 µL / h flush for 1 minute in the air and liquid channels.</td></tr>
<tr><td>3</td><td>Immediately aspirate the outlet reservoir corresponding to the air channel completely.</td></tr>
<tr><td>4</td><td>Re-introduce the liquid lock in the inlet and outlet corresponding to the air channel, then proceed with normal flow.</td></tr>
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