Steps to Diluting Methacholine Chloride

**ATS “Short” - Quadrupling Doses**

**Dilution Sequence Protocol for Provocholine 100 mg/vial (US NDC № 64281-100-06)**

### Getting Started

1. Attach labels to sterile empty vials.
2. Wipe down the stoppers of the Provocholine vial, diluent vial, and sterile empty vials with alcohol prep pads.
3. Label two (2) appropriately sized syringes (one for Provocholine, one for diluent), and attach needles to each.

### Preparing Vial A

1. **16 mg/mL Solution**
   - Using the diluent needle and syringe, draw 6.25 mL of diluent and transfer the 6.25 mL of diluent to the Provocholine 100 mg vial.
   - Shake well.

### Preparing Vial B

1. **4 mg/mL Solution**
   - Using the Provocholine needle and syringe, draw 3 mL of solution from Vial A and transfer to Vial B.
   - Using the diluent needle and syringe, draw 9 mL of diluent and transfer to Vial B.
   - Shake well.

### Preparing Vial C

1. **1 mg/mL Solution**
   - Using the Provocholine needle and syringe, draw 3 mL of solution from Vial B and transfer to Vial C.
   - Using the diluent needle and syringe, draw 9 mL of diluent and transfer to Vial C.
   - Shake well.

### Preparing Vial D

1. **0.25 mg/mL Solution**
   - Using the Provocholine needle and syringe, draw 3 mL of solution from Vial C and transfer to Vial D.
   - Using the diluent needle and syringe, draw 9 mL of diluent and transfer to Vial D.
   - Shake well.
Preparation of Vial E
0.0625 mg/mL Solution

1. Using the Provocholine needle and syringe, draw 3 mL of solution from Vial D and transfer to Vial E.

2. Using the diluent needle and syringe, draw 9 mL of diluent and transfer to Vial E.

3. Shake well.

**Diluent Check Sheet and Control Record**

PROVOCHOLINE DILUTIONS FOR CHALLENGE TESTING

- Date ____________________
- Provocholine (see label on vial)
- Diluent ________________
- Prepared by ________________
- Lot Number ________________
- Checked by ________________
- Expiration Date ________________

<table>
<thead>
<tr>
<th>Take Provocholine</th>
<th>Add diluent (Shake well!)</th>
<th>Obtain dilution</th>
<th>Vial name</th>
<th>Initial / Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provocholine 100 mg</td>
<td>6.25 mL</td>
<td>16 mg/mL</td>
<td>Vial A - 16 mg/mL</td>
<td></td>
</tr>
<tr>
<td>3 mL from Vial A</td>
<td>9 mL</td>
<td>4 mg/mL</td>
<td>Vial B - 4 mg/mL</td>
<td></td>
</tr>
<tr>
<td>3 mL from Vial B</td>
<td>9 mL</td>
<td>1 mg/mL</td>
<td>Vial C - 1 mg/mL</td>
<td></td>
</tr>
<tr>
<td>3 mL from Vial C</td>
<td>9 mL</td>
<td>0.25 mg/mL</td>
<td>Vial D - 0.25 mg/mL</td>
<td></td>
</tr>
<tr>
<td>3 mL from Vial D</td>
<td>9 mL</td>
<td>0.0625 mg/mL</td>
<td>Vial E - 0.0625 mg/mL</td>
<td></td>
</tr>
</tbody>
</table>

**Storage Instructions**

- Provocholine powder should be stored at 59º to 86ºF (15º to 30ºC).
- Dilutions A through E (16 mg/mL through 0.0625 mg/mL) should be stored at 36º to 46ºF (2º to 8ºC) in a refrigerator for no more than 2 weeks.
- Freezing does not affect the stability of dilutions A through E (16 mg/mL through 0.0625 mg/mL).

For technical support, please contact us at 519.751.3602 | 1.800.287.7686