Steps to Diluting Methacholine Chloride
US Package Insert - Single Patient

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.

Preparation of Vials

Preparing Vial A

25 mg/mL Solution

1. Using the diluent needle and syringe, draw 4 mL of diluent and transfer the 4 mL of diluent to the Provocholine 100 mg vial.
2. Shake well.

Preparing Vial B

10 mg/mL Solution

1. Using the Provocholine needle and syringe, draw 1 mL of solution from Vial A and transfer to Vial B.
2. Using the diluent needle and syringe, draw 1.5 mL of diluent and transfer to Vial B.
3. Shake well.

Preparing Vial C

2.5 mg/mL Solution

1. Using the Provocholine needle and syringe, draw 1 mL of solution from Vial A and transfer to Vial C.
2. Using the diluent needle and syringe, draw 9 mL of diluent and transfer to Vial C.
3. Shake well.

Preparing Vial D

0.25 mg/mL Solution

1. Using the Provocholine needle and syringe, draw 1 mL of solution from Vial C and transfer to Vial D.
2. Using the diluent needle and syringe, draw 9 mL of diluent and transfer to Vial D.
3. Shake well.
Preparing Vial E
0.025 mg/mL Solution

1. Using the Provocholine needle and syringe, draw 1 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.

NOTE
1. Vial E must be prepared on the day of the challenge test.
2. Transfer all dilutions (in vials A through E) to nebulizer through the 0.22 µm sterile bacterial retentive filter (Millex GV®).*

*Do not attach filter until AFTER solution has been drawn into syringe.

Dilution Check Sheet and Control Record

PROVOCHELINE DILUTIONS FOR CHALLENGE TESTING

<table>
<thead>
<tr>
<th>Date______________</th>
<th>Prepared by______________</th>
<th>Checked by______________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provocholine (see label on vial)</td>
<td>Lot Number______________</td>
<td>Expiration Date______________</td>
</tr>
<tr>
<td>Diluent______________</td>
<td>Lot Number______________</td>
<td>Expiration Date______________</td>
</tr>
</tbody>
</table>

<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>4 mL</td>
<td>25 mg/mL</td>
<td>Vial A - 25 mg/mL</td>
<td></td>
</tr>
<tr>
<td>1 mL from Vial A</td>
<td>1.5 mL</td>
<td>10 mg/mL</td>
<td>Vial B - 10 mg/mL</td>
<td></td>
</tr>
<tr>
<td>1 mL from Vial A</td>
<td>9 mL</td>
<td>2.5 mg/mL</td>
<td>Vial C - 2.5 mg/mL</td>
<td></td>
</tr>
<tr>
<td>1 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>1 mL from Vial D</td>
<td>9 mL</td>
<td>0.025 mg/mL</td>
<td>Vial E - 0.025 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 D (25 mg/mL through 0.25 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 D (25 mg/mL through 0.25 mg/mL).
- Vial E (0.025 mg/mL) must be prepared on the day of the challenge test.

For technical support, please contact us at 519.751.3602 | 1.800.287.7686