Mixing Methacholine Dilutions for Bronchoprovocation Challenge Testing
Overview

1. Gathering Necessary Equipment
2. Special Precautions
3. Reconstituting Provocholine
1 Supplies

- 100 mg vial of Provocholine
1 Supplies

- Diluent options
  - 0.9% Saline
  - 0.9% Saline with 0.4% Phenol (pH 7.0)*
- Sterile empty vials*
- Syringes and needles

* Available for purchase from Methapharm, Inc.
1 Supplies

- 0.22 μm sterile bacterial-retentive filter*
- Alcohol preparation pads
- Green labels (included)

* Available for purchase from Methapharm, Inc.
2 Reconstituting Precautions

- Please refer to the Provocholine package insert for full instructions and safety precautions
2 Reconstituting Precautions

- Accurate sterile technique is essential to obtain appropriate test results while maintaining patient safety.
2 Precautions – Green Labels

- Fill in all information on the labels
- Include 14 day expiration on Vials A-D
- Vial E should be prepared the day of testing
2 Precautions – Green Labels

<table>
<thead>
<tr>
<th>Precaution</th>
<th>Description</th>
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<tbody>
<tr>
<td>Strength</td>
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<tr>
<td>Diluent</td>
<td>Blank for input</td>
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<td>Blank for input</td>
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<tr>
<td>Exp. Date:</td>
<td>Blank for input</td>
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<tr>
<td>Dilution Strength</td>
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<tr>
<td>Lot number</td>
<td>Blank for input</td>
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<tr>
<td>Initials of person</td>
<td>Blank for input</td>
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<tr>
<td>performing dilution</td>
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- **Type of Diluent Used**: Blank for input
- **Preparation Date**: Blank for input
- **Expiration Date**: Blank for input
- **Dilution Strength**: Blank for input
- **Lot number**: Blank for input
- **Initials of person performing dilution**: Blank for input
## Dosing Protocols

<table>
<thead>
<tr>
<th>US Package Insert (mg/mL)</th>
<th>ATS Short (mg/mL)</th>
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<tbody>
<tr>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
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<tr>
<td>2.5</td>
<td>1</td>
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<tr>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>0.025</td>
<td>0.0625</td>
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<tr>
<td>Diluent</td>
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Step 1

Attach the completed green labels to the sterile empty vials.
Step 2

Wipe the top of the Provocholine vial, the diluent vial and the sterile empty vials with alcohol preparation pads.
Step 3

Label two syringes, one for the Provocholine and one for the diluent.
Step 4 – Package Insert Dilutions

Using the Diluent syringe, withdraw 4 mL of diluent and inject it into the Provocholine vial creating a stock solution. Shake well.

This solution contains 25 mg/mL of methacholine chloride.
Step 5 – Package Insert Dilutions

Using the Provocholine vial as your stock solution, withdraw 1 mL of the 25 mg/mL solution with the Provocholine syringe and add it to the vial labeled Vial B.
Step 5 – Package Insert Dilutions

Using the Diluent syringe, add 1.5 mL of diluent to the same vial. Shake well.

Vial B will contain a 10 mg/mL solution of methacholine chloride.
Step 6 – Package Insert Dilutions

Using the Provocholine vial as your stock solution, withdraw 1 mL of the 25 mg/mL solution with the Provocholine syringe and add it to the vial labeled Vial C.
Step 6 – Package Insert Dilutions

Using the Diluent syringe, add 9 mL of diluent to the same vial. Shake well.

Vial C will contain a 2.5 mg/mL solution of methacholine chloride.
Step 7 – Package Insert Dilutions

From here, we will perform a serial dilution to make the remaining two concentrations.

Using the Provocholine syringe, withdraw 1 mL from Vial C (2.5 mg/mL) and add it to the vial labeled Vial D.
Step 7 – Package Insert Dilutions

Using the Diluent syringe, add 9 mL of diluent to the same vial. Shake well.

Vial D will contain a 0.25 mg/mL solution of methacholine chloride.
Repeat the same process for Vial E the day of the test, withdrawing 1 mL from Vial D (0.25 mg/mL) and adding 9 mL of diluent.

Vial E will contain a 0.025 mg/mL solution of methacholine chloride.
Step 4 – ATS Short Dilutions

Using the Diluent syringe, withdraw 6.25 mL of diluent and inject it into the Provocholine vial. Shake well.

This solution contains 16 mg/mL of methacholine chloride.
Step 5 – ATS Short Dilutions

For the ATS Short Dilutions you will perform a serial dilution using the previous dilution as the stock solution at each step.

Using the Provocholine vial, withdraw 3 mL of the 16 mg/mL solution using the Provocholine syringe and add it to the vial labeled Vial B.
Step 5 – ATS Short Dilutions

Using the Diluent syringe, add 9 mL of diluent to the same vial. Shake well.

Vial B will contain a 4 mg/mL solution of methacholine chloride.
Step 6 – ATS Short Dilutions

Using the previous vial (Vial B), withdraw 3 mL of the 4 mg/mL solution using the Provocholine syringe and add it to the vial labeled Vial C.
Step 6 – ATS Short Dilutions

Using the Diluent syringe, add 9 mL of diluent to the same vial. Shake well.

Vial C will contain a 1 mg/mL solution of methacholine chloride.
Step 7 – ATS Short Dilutions

Using the Provocholine syringe, withdraw 3 mL from Vial C (1 mg/mL) and add it to the vial labeled Vial D. Add 9 mL of diluent to the same vial. Shake well.

Vial D will contain a 0.25 mg/mL solution of methacholine chloride.
Step 8 – ATS Short Dilutions

Repeat the same process for vial E the day of the test, withdrawing 3 mL from Vial D (0.25 mg/mL) and adding 9 mL of diluent.

Vial E will contain a 0.0625 mg/mL solution of methacholine chloride.
3 Storage of Dilutions

- Refrigerate reconstituted solutions at 36°C to 46°F (2°C to 8°C) for up to 2 weeks for vials A-D
- Vial E must be prepared on the day of testing
- Freezing does not affect stability of the dilutions
## Summary

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Questions or Concerns

Contact Methapharm at 1-800-287-7686