
PROVOCHOLINE®

(methacholine chloride)

Dilution Sequence- Multiple Patient Testing

(2 – 5 patients: requires 2 vials of Provocholine®)

The Provocholine® Challenge Test



Provocholine® 100 mg/vial (US NDC no. 64281-0100-06)

While Methapharm Inc. uses reasonable efforts to include accurate and up to date information in this presentation it makes no warranties or representations with respect to the accuracy, currency or completeness of the contents of this presentation.

Introduction

Dilution Sequence Protocol

- These instructions are prepared according to the United States FDA approved Package Insert for Provocholine[®] (NDC 64281-0100-06) for multiple patient testing.
- The following are step-by-step instructions to dilute 2 100 mg vials of Provocholine[®] into the five (5) requisite concentrations for use in a bronchoprovocation challenge test
- **Please refer to the Package Insert for full instructions and safety precautions**
- Accurate sterile mixing is essential for the accuracy of the test results and to maintain patient safety
- Only trained individuals should mix and label Provocholine[®] solutions
- The following protocol is used to prepare Provocholine[®] solutions for testing multiple patients.

Important Notes

- Do not inhale powder during preparation of dilutions
- Do not handle Provocholine[®] if you have asthma or hay fever
- Provocholine[®] dilutions should be mixed by a pharmacist or other well-trained individual using sterile technique
- All vials should be labeled using the green labels provided, filling in the appropriate Lot Number, concentration, Diluent, preparation, initials of person preparing, and expiration dates.
- To reduce back pressure, vent vials with an extra needle as needed
- All dilutions should be made with:
 - 0.9% NaCl containing 0.4% phenol (pH 7.0)
 - Sterile USP Type I borosilicate glass vials
- When transferring solution from each vial (at least 2 mL) to a nebulizer use a sterile bacterial-retentive filter (porosity 0.22 µm)

Supplies Required

Supplies required for the dilution of two (2) vials of Provocholine® 100 mg

Quantity	Description
2	100 mg/vials of Provocholine®
1	100 mL 0.9% NaCl with 0.4% Phenol (pH 7.0)
4	10 mL Sterile empty USP Type I borosilicate glass vials
2	10 mL Syringes*
2	20 Gauge, 1" Syringe Needles*
1	Millex GS 0.22 µm filter (Millipore)*
7	Alcohol Preparation Pads*
1 set	Green labels for vials (provided with Provocholine®)
X	Set of Directions
X	Provocholine® Dilution Sequence Check Sheet and Control Record
X	Package Insert for Provocholine®

*Quantity of supplies subject to facility protocol

Overview of the Dilution Process

TAKE Provocholine® (Methacholine Chloride U.S.P. Powder for Inhalation)	ADD NaCl 0.9% with 0.4% phenol (Shake well!)	OBTAIN DILUTION	VIAL NAME
Provocholine® 100 mg x 2	4.0 mL	25 mg/mL	VIALS A₁ & A₂ - 25 mg/mL
3 mL from vial A₁	4.5 mL	10 mg/mL	VIAL B - 10 mg/mL
1 mL from vial A₂	9.0 mL	2.5 mg/mL	VIAL C - 2.5 mg/mL
1 mL from vial C	9.0 mL	0.25 mg/mL	VIAL D - 0.25 mg/mL
1 mL from vial D	9.0 mL	0.025 mg/mL	VIAL E - 0.025 mg/mL

Getting Started – Green Labels

- Fill in all information on green labels provided
- Include 14 day expiration date on vials A₁ & A₂ - D

The diagram shows a green label for Provocholine with the following fields and callouts:

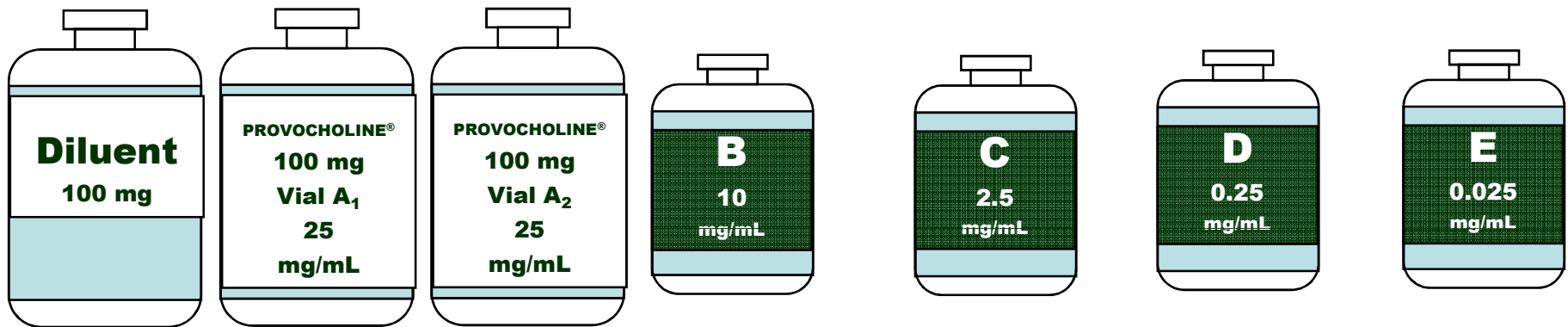
- Type of Diluent Used**: Points to the Strength field.
- Dilution Strength**: Points to the Diluent field.
- Preparation Date**: Points to the Prep. Date field.
- Expiry Date (2 weeks after preparation)**: Points to the Exp. Date field.
- Lot Number**: Points to the Mfr. Lot No. field.
- Initials of Person Performing Dilution**: Points to the Initials field.

The label itself contains the following text:

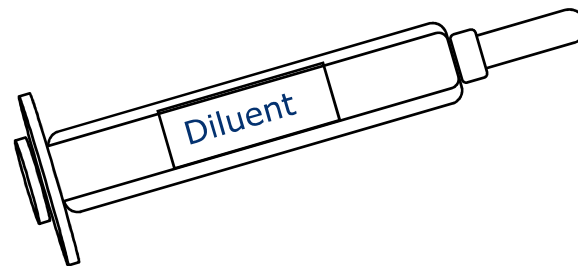
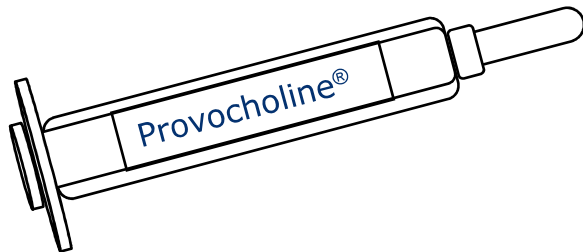
Provocholine[®]
Mfr. Lot No. **P-**
Initials
www.methapharm.com

Getting Started

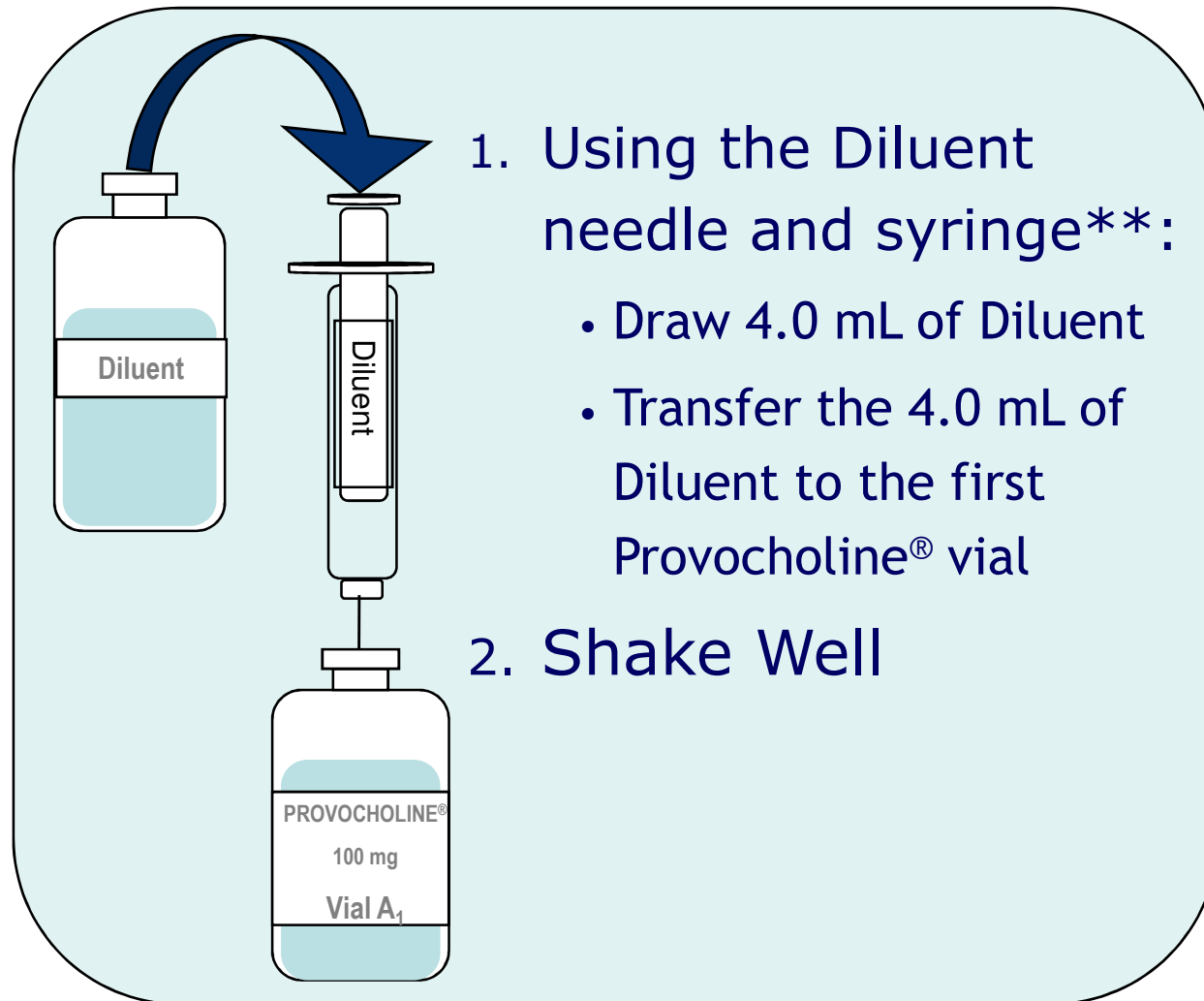
1. Attach green labels to sterile empty vials
2. Wipe down the plastic tops of the Sterile Empty vials, Provocholine[®], and Saline vials with alcohol prep pads:



3. Label two (2) 10 mL syringes, (one for Provocholine[®] and one for Diluent) and attach needles to each



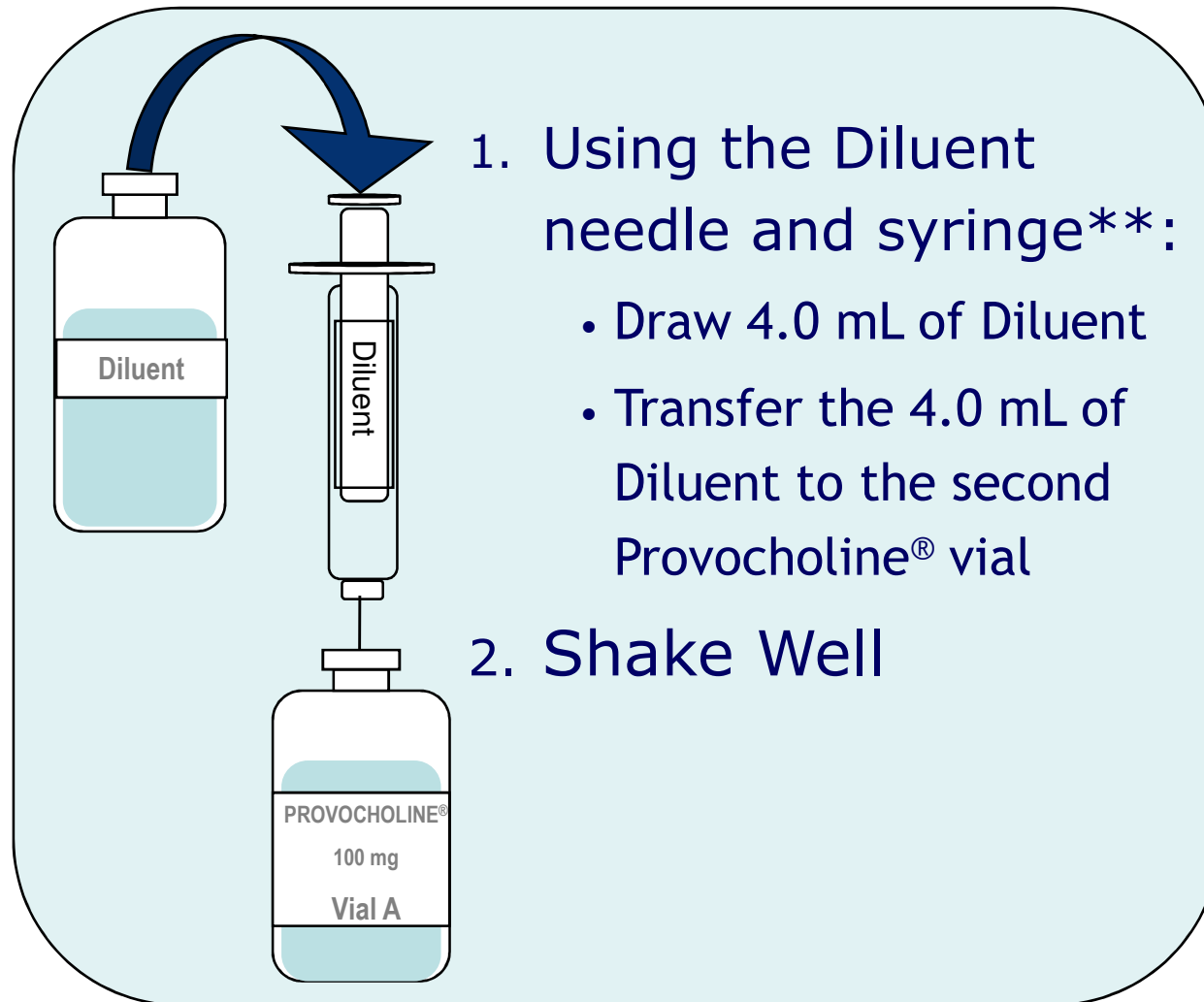
Step I: Preparing Vial A₁ - Provocholine® 25 mg/mL Solution



After Completing Step I:

Vial A₁ contains 4.0 mL of Provocholine® solution @ 25 mg/mL

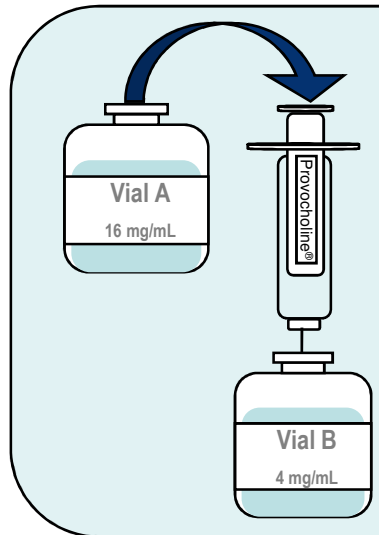
Step II: Preparing Vial A₂ - Provocholine® 25 mg/mL Solution



After Completing Step I:

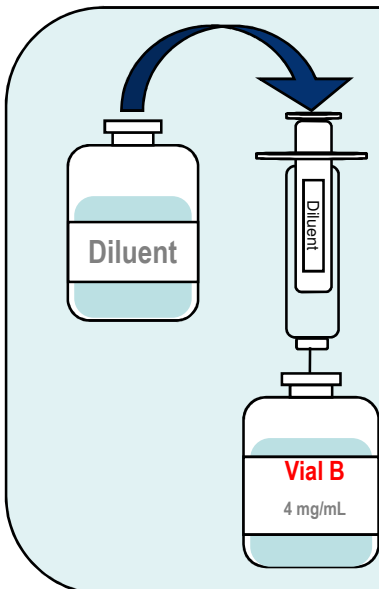
Vial A₂ contains 4.0 mL of Provocholine® solution @ 25 mg/mL

Step III: Preparing Vial B - Provocholine® 10 mg/mL solution



The diagram shows a vial labeled 'Vial A' containing a 16 mg/mL solution. A syringe with a 'Provocholine' needle is shown drawing liquid from Vial A. An arrow indicates the transfer of liquid to a second vial labeled 'Vial B' containing a 4 mg/mL solution.

1. Using the Provocholine® needle and syringe:
 - Draw 3 mL from Vial A₁
 - Transfer the 3 mL to Vial B



The diagram shows a vial labeled 'Diluent' and a syringe with a 'Diluent' needle. An arrow indicates the transfer of liquid from the Diluent vial to a vial labeled 'Vial B' containing a 4 mg/mL solution.

2. Using the Diluent needle and syringe:
 - Add 4.5 mL of Diluent to Vial B
2. Shake well

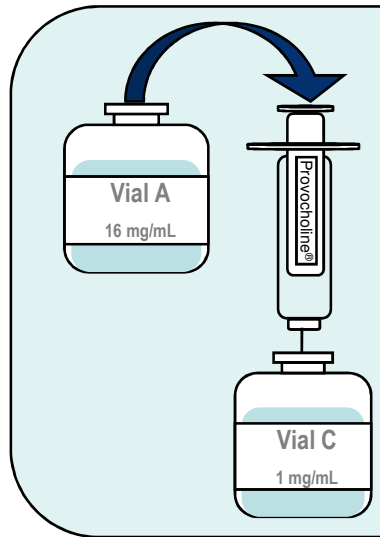
After Completing Step II:

Vial A₁ contains 1 mL of Provocholine® solution @ 25 mg/mL

- and -

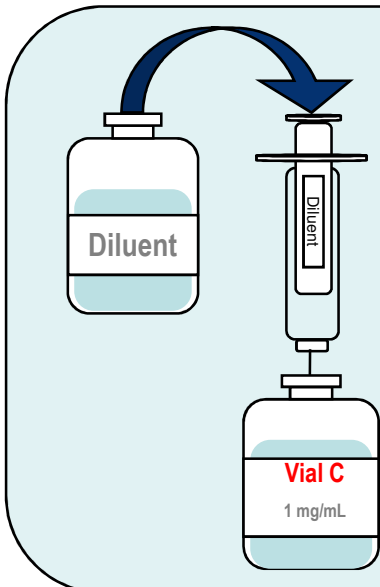
Vial B contains 7.5 mL of Provocholine® solution @ 10 mg/mL

Step IV: Preparing Vial C - Provocholine® 2.5 mg/mL solution



The diagram shows a vial labeled 'Vial A' containing '16 mg/mL' of Provocholine. A syringe with a 'Provocholine®' needle is shown drawing 1 mL from Vial A. An arrow indicates the transfer of this 1 mL into a vial labeled 'Vial C' which contains '1 mg/mL' of solution.

1. Using the Provocholine® needle and syringe,
 - Draw 1 mL from Vial A₂
 - Transfer the 1 mL to Vial C



The diagram shows a vial labeled 'Diluent' being used to fill a syringe. An arrow indicates the addition of 9 mL of diluent to Vial C. The final vial is labeled 'Vial C' and contains '1 mg/mL' of solution.

2. Using the Diluent needle and syringe:
 - Add 9 mL of Diluent to Vial C
3. Shake well

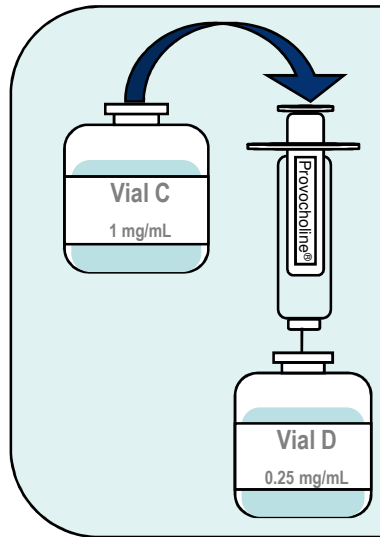
After Completing Step III:

Vial A₂ contains 3 mL of Provocholine® solution @ 4 mg/mL

-and-

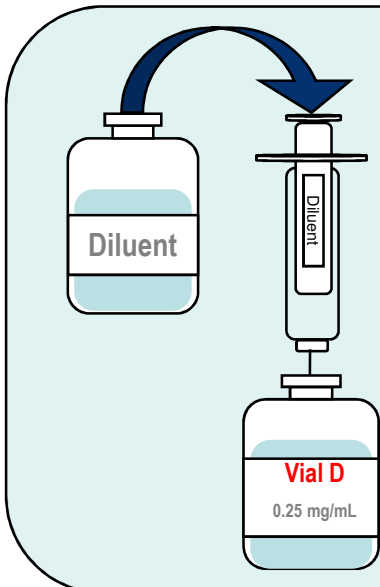
Vial C contains 10 mL of Provocholine® solution @ 2.5 mg/mL

Step V: Preparing Vial D - Provocholine[®] 0.25 mg/mL solution



The diagram shows a vial labeled 'Vial C' containing '1 mg/mL' of Provocholine. A syringe with a 'Provocholine[®]' needle is shown drawing liquid from Vial C. An arrow indicates the transfer of liquid to a vial labeled 'Vial D' which contains '0.25 mg/mL'.

1. Using the Provocholine[®] needle and syringe:
 - Draw 1 mL from Vial C
 - Transfer the 1 mL to Vial D



The diagram shows a vial labeled 'Diluent' being drawn into a syringe with a 'Diluent' needle. An arrow indicates the transfer of liquid to a vial labeled 'Vial D' which contains '0.25 mg/mL'.

2. Using the Diluent needle and syringe:
 - Add 9 mL of Diluent to Vial D
3. Shake well

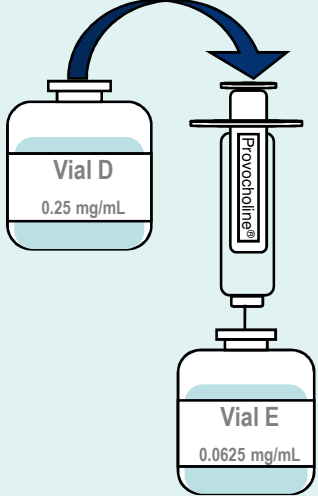
After Completing Step IV:

Vial C contains 9 mL of Provocholine[®] solution @ 2.5 mg/mL

-and-

Vial D contains 10 mL of Provocholine[®] solution 0.25 mg/mL

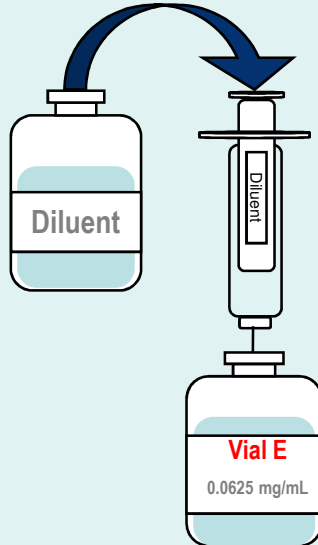
Step VI: Preparing Vial E - Provocholine® 0.025 mg/mL solution (Day of Test)



The diagram shows a vial labeled 'Vial D' containing '0.25 mg/mL' of Provocholine. A syringe with a 'Provocholine®' needle is shown drawing liquid from Vial D. An arrow points from the syringe to a vial labeled 'Vial E' containing '0.0625 mg/mL'.

***Vial E must only be prepared on the day of the test**

1. Using the Provocholine® needle and syringe:
 - Draw 1 mL from Vial D,
 - Transfer the 1 mL to Vial E



The diagram shows a vial labeled 'Diluent' and a syringe with a 'Diluent' needle. An arrow points from the syringe to a vial labeled 'Vial E' containing '0.0625 mg/mL'.

2. Using the Diluent needle and syringe:
 - Add 9 mL of Diluent to Vial E
3. Shake well

After Completing Step V:

Vial D

contains 9 mL of Provocholine® solution @ 0.25 mg/mL

-and-

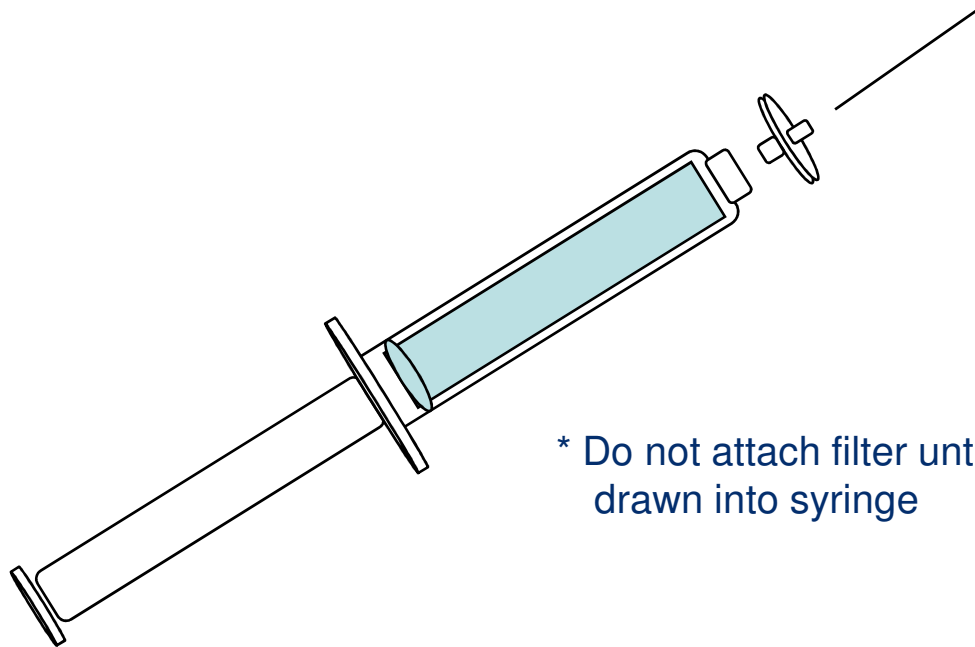
Vial E

contains 10 mL of Provocholine® solution @ 0.025 mg/mL

Final Step: Day of the Test

After Preparing Vial E:

- Aseptically attach a 0.22 μm bacterial retentive filter (Millex GS[®]) to a 10 mL syringe*
- Transfer all dilutions (in vials A₁ & A₂ through E) to nebuliser through the 0.22 μm bacterial retentive filter (Millex GS[®])



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

Storage Instructions

- Dilutions A_1 & A_2 through D (25 mg/mL through 0.25 mg/mL) should be stored at 36° to 45°F (2° to 8°C) in a refrigerator for no more than 2 weeks
- Vial E (0.025 mg/mL) must be prepared on the day of the challenge
- Unreconstituted powder should be stored at 59° to 86 °F (15° to 30°C)
- Freezing does not affect the stability of dilutions A_1 & A_2 through D (25 mg/mL through 0.25 mg/mL)

Summary of Directions

Procedure:

1. Attach 2 needles to two (2), 10 mL syringes and label one for Provocholine® and one for Diluent.
2. Remove plastic covers from the Provocholine® and Diluent vials and line up all sterile empty vials.
3. Fill in information and attach green labels to the sterile empty vials.
4. Wipe off all plastic vial stoppers for Provocholine®, Diluent and sterile empty vials.
5. Using the Diluent needle and syringe, withdraw 4.0 mL of Diluent and insert into the first Provocholine® 100mg vial. SHAKE WELL. This produces 4.0 mL of Provocholine® 25mg/mL also known as **VIAL A₁ – 25 MG/ML.**
6. Using the Diluent needle and syringe, withdraw 4.0 mL of Diluent and insert into the second Provocholine® 100mg vial. SHAKE WELL. This produces 4.0 mL of Provocholine® 25mg/mL also known as **VIAL A₂ – 25 MG/ML.**
7. Using the Provocholine® needle and syringe, remove 3 mL from VIAL A₁ and transfer to the vial labelled VIAL B – 10 MG/ML. Using the Diluent needle and syringe, add 4.5 mL Diluent. Shake well. **THIS IS VIAL B – 10 MG/ML.**
8. Using the Provocholine® needle and syringe, remove 1 mL from VIAL A₂ and transfer to the vial labelled VIAL C – 2.5 MG/ML. Using the Diluent needle and syringe, add 9 mL of Diluent. Shake well. **THIS IS VIAL C – 2.5 MG/ML.**
9. Using the Provocholine® needle and syringe, remove 1 mL from VIAL C, and transfer to the vial labelled VIAL D – 0.25 MG/ML. Using the Diluent needle and syringe, add 9 mL of Diluent. Shake well. **THIS IS VIAL D – 0.25 MG/ML.**
10. Using the Provocholine® needle and syringe, remove 3 mL from VIAL D – 0.25 MG/ML and transfer to the vial labelled VIAL E – 0.025 MG/ML. Using the Diluent needle and syringe, add 9 mL of Diluent. Shake well. **THIS IS VIAL E – 0.025 MG/ML.**

NOTE

- A sterile bacterial-retentive filter (porosity 0.22 µm) should be used when transferring a solution from each vial to a nebulizer.
- To avoid back pressure when injecting the contents of a syringe into a vial, you may wish to vent the vial with another needle.

Dilution Check Sheet and Control Record

PROVOCHOLINE® DILUTIONS FOR CHALLENGE TEST

Date: _____ Prepared by: _____ Checked by: _____

Provocholine® (see label on bottle): _____ Expiration Date: _____

Diluent: _____ Expiration Date: _____

0.22 micron Filter: _____ Expiration Date: _____

TAKE Provocholine® (Methacholine Chloride U.S.P. Powder for Inhalation)	ADD NaCl 0.9% with 0.4% phenol (Shake well!)	OBTAIN DILUTION	VIAL NAME	COMPLETED
Provocholine® 100 mg x 2	4.0 mL	25 mg/mL	VIALS A1 & A2 - 25 mg/mL	
3 mL from viaL A1	4.5 mL	10 mg/mL	VIAL B - 10 mg/mL	
1 mL from viaL A2	9.0 mL	2.5 mg/mL	VIAL C - 2.5 mg/mL	
1 mL from viaL C	9.0 mL	0.25 mg/mL	VIAL D - 0.25 mg/mL	
1 mL from viaL D	9.0 mL	0.025 mg/mL	VIAL E - 0.025 mg/mL	