

# PROVOCHOLINE®

(methacholine chloride)

## Dilution Sequence Protocol for The Provocholine® Challenge Test



Provocholine® 100 mg/vial – “ATS – Long”

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.

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## Introduction

### Dilution Sequence Protocol

- These instructions are for the preparation prepared according to the ATS Guidelines for Methacholine and Exercise Challenge published in 2000 by the American Thoracic Society. This protocol is commonly known at the “ATS Long” <http://www.thoracic.org/sections/publications>
- The following are step-by-step instructions to dilute a 100 mg vial of Provocholine® into the 10 (ten) 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 methacholine solutions
- The following protocol is used to prepare a 100 mg vial of Provocholine® for testing a single patient only

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## 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:
  - Diluent
  - Sterile, empty 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 a single (1) vial of Provocholine® 100 mg

Quantity	Description
1	100 mg/vial of Provocholine®
1	100 mL Diluent
9	10 mL Sterile empty USP Type I borosilicate glass vials
2	10 mL Syringes* **
2	20 Gauge, 1" Syringe Needles*
1	Millex GV 0.22 µm filter (Millipore)*
12	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

\*\*Please note that the dilution in Step I requires that you draw 6.25 mL of Diluent, therefore you should use appropriately graduated syringes to accurately draw the Diluent (i.e. use an additional 1 mL syringe for Step I)



## Overview of the Dilution Process

TAKE Provocholine® (Methacholine Chloride U.S.P. Powder for Inhalation)	ADD Diluent (Shake well!!)	OBTAIN DILUTION	VIAL NAME
Provocholine® 100 mg	6.25 mL	16 mg/mL	VIAL A - 16 mg/mL
3 mL from vial A	3 mL	8 mg/mL	VIAL B - 8 mg/mL
3 mL from vial B	3 mL	4 mg/mL	VIAL C - 4 mg/mL
3 mL from vial C	3 mL	2 mg/mL	VIAL D - 2 mg/mL
3 mL from vial D	3 mL	1 mg/mL	VIAL E - 1 mg/mL
3 mL from vial E	3 mL	0.5 mg/mL	VIAL F - 0.5 mg/mL
3 mL from vial F	3 mL	0.25 mg/mL	VIAL G - 0.25 mg/mL
3 mL from vial G	3 mL	0.125 mg/mL	VIAL H - 0.125 mg/mL
3 mL from vial H	3 mL	0.0625 mg/mL	VIAL I - 0.0625 mg/mL
3 mL from vial I	3 mL	0.031 mg/mL	VIAL J - 0.031 mg/mL



## Getting Started – Green Labels

- Fill in all information on green labels provided
- Include 14 day expiration date on vials A - J

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

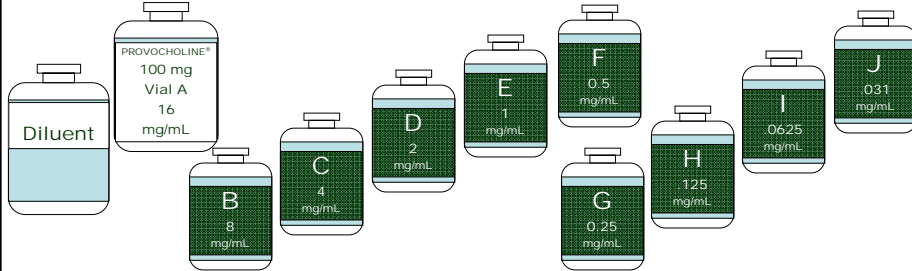
- Type of Diluent Used**: Points to the 'Diluent:' field.
- Dilution Strength**: Points to the 'Strength:' field.
- Preparation Date**: Points to the 'Prep. Date:' field.
- Expiry Date (2 weeks after preparation)**: Points to the 'Exp. Date:' field.
- Lot Number**: Points to the 'Mfr. Lot No.' field.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

The label also contains the text: Provocholine®, Mfr. Lot No., P-, Initials, and www.methapharm.com.

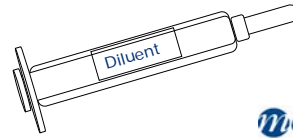


## Getting Started

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:

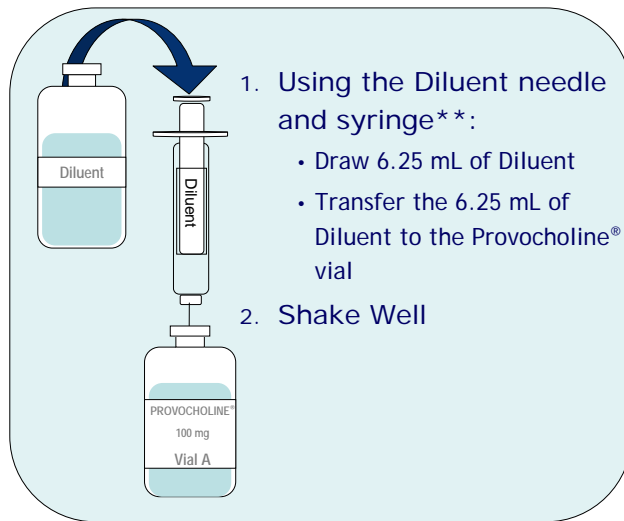


3. Label two (2) 10 mL syringes, (one for Provocholine® and one for Diluent) and attach needles to each



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## Step I: Preparing Vial A - Provocholine® 16 mg/mL Solution



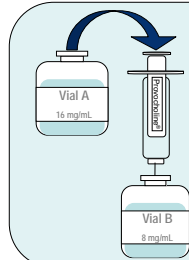
1. Using the Diluent needle and syringe\*\*:
  - Draw 6.25 mL of Diluent
  - Transfer the 6.25 mL of Diluent to the Provocholine® vial
2. Shake Well

**After Completing Step I:**  
**Vial A**  
**contains 6.25 mL**  
**of Provocholine®**  
**Solution @**  
**16 mg/mL**

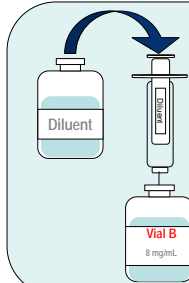
\*\*Please note that the dilution in Step I requires that you draw 6.25 mL of Diluent, therefore you should use appropriately graduated syringes to accurately draw the Diluent (i.e. use an additional 1 mL syringe for Step I)

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## Step II: Preparing Vial B - Provocholine® 8 mg/mL solution



1. Using the Provocholine® needle and syringe:
  - Remove 3 mL from Vial A,
  - Transfer the 3 mL to Vial B



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

### **After Completing Step II:**

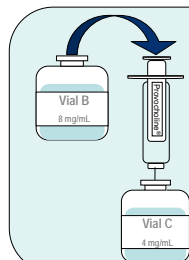
**Vial A**  
contains 3.25mL  
of Provocholine®  
solution @  
16 mg/mL

**-and-**

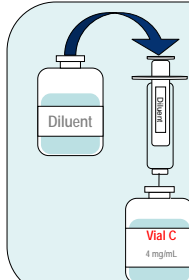
**Vial B**  
contains 6 mL  
of Provocholine®  
solution @  
8 mg/mL

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## Step III: Preparing Vial C - Provocholine® 4 mg/mL solution



1. Using the Provocholine® needle and syringe,
  - Remove 3 mL from Vial B
  - Transfer the 3 mL to Vial C



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

### **After Completing Step III:**

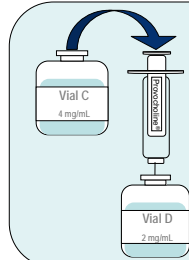
**Vial B**  
contains 3 mL  
of Provocholine®  
solution @  
8 mg/mL

**-and-**

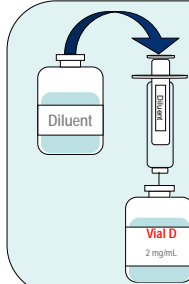
**Vial C**  
contains 6 mL  
of Provocholine®  
solution @  
4 mg/mL

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## Step IV: Preparing Vial D - Provocholine® 2 mg/mL solution



1. Using the Provocholine® needle and syringe:
  - Remove 3 mL from Vial C
  - Transfer the 3 mL to Vial D



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

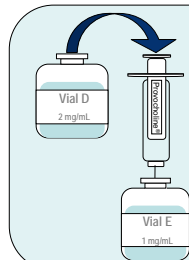
### **After Completing Step IV:**

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

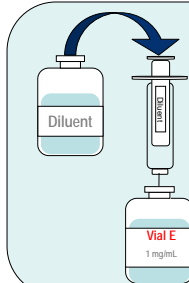
**- and -**

**Vial D contains 6 mL of Provocholine® solution @ 2 mg/mL**

## Step V: Preparing Vial E - Provocholine® 1 mg/mL solution



1. Using the Provocholine® needle and syringe:
  - Remove 3 mL from Vial D
  - Transfer the 3 mL to Vial E



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

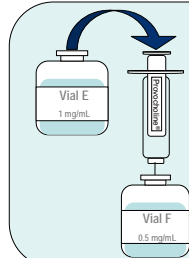
### **After Completing Step V:**

**Vial D contains 3 mL of Provocholine® solution @ 2 mg/mL**

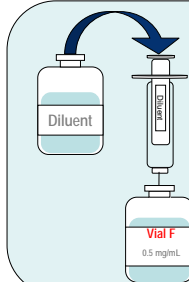
**-and-**

**Vial E contains 6 mL of Provocholine® solution @ 1 mg/mL**

## Step VI: Preparing Vial F - Provocholine® 0.5 mg/mL solution



1. Using the Provocholine® needle and syringe:
  - Remove 3 mL from Vial E
  - Transfer the 3 mL to Vial F



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

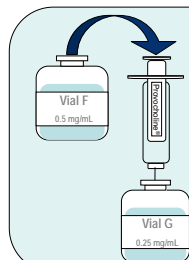
### **After Completing Step VI:**

**Vial E** contains 3 mL of Provocholine® solution @ 1 mg/mL

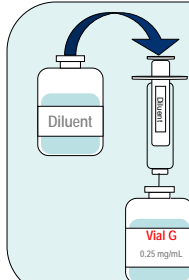
-and-

**Vial F** contains 6 mL of Provocholine® solution @ 0.5 mg/mL

## Step VII: Preparing Vial G - Provocholine® 0.25 mg/mL solution



1. Using the Provocholine® needle and syringe:
  - Remove 3 mL from Vial F
  - Transfer the 3 mL to Vial G



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

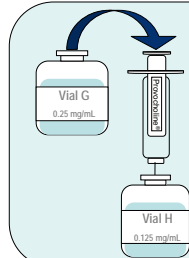
### **After Completing Step VII:**

**Vial F** contains 3 mL of Provocholine® solution @ 0.5 mg/mL

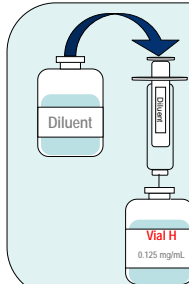
-and-

**Vial G** contains 6 mL of Provocholine® solution @ 0.25 mg/mL

## Step VIII: Preparing Vial H - Provocholine® 0.125 mg/mL solution



1. Using the Provocholine® needle and syringe:
  - Remove 3 mL from Vial G
  - Transfer the 3 mL to Vial H



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

### **After Completing Step VIII:**

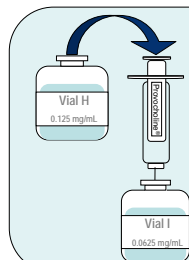
**Vial G contains 3 mL of Provocholine® solution @ 0.25 mg/mL**

**-and-**

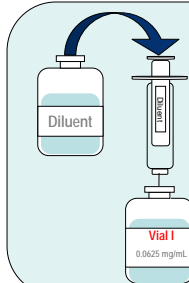
**Vial H contains 6 mL of Provocholine® solution @ 0.125 mg/mL**



## Step IX: Preparing Vial I - Provocholine® 0.0625 mg/mL solution



1. Using the Provocholine® needle and syringe:
  - Remove 3 mL from Vial H
  - Transfer the 3 mL to Vial I



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

### **After Completing Step IX:**

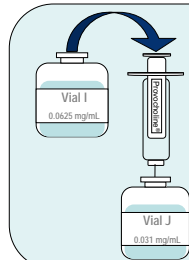
**Vial H contains 3 mL of Provocholine® solution @ 0.125 mg/mL**

**-and-**

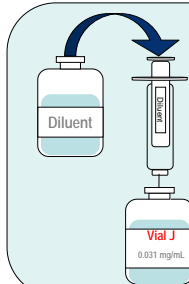
**Vial I contains 6 mL of Provocholine® solution @ 0.0625 mg/mL**



## Step X: Preparing Vial J - Provocholine® 0.031 mg/mL solution



1. Using the Provocholine® needle and syringe:
  - Remove 3 mL from Vial I,
  - Transfer the 3 mL to Vial J



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

### **After Completing Step X:**

**Vial I contains 3 mL of Provocholine® solution @ 0.0625 mg/mL**

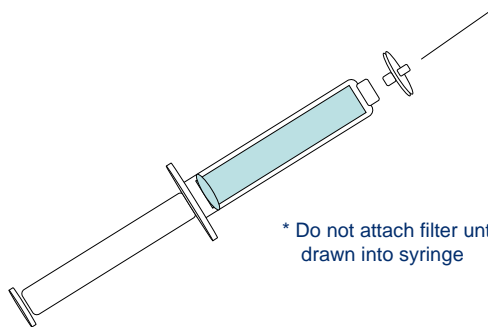
**-and-**

**Vial J contains 6 mL of Provocholine® solution @ 0.031 mg/mL**



## Final Step: Day of the Test

- Aseptically attach a 0.22 µm bacterial retentive filter (Millex GV®) to a 10 mL syringe\*
- Transfer all dilutions (in vials A through J) to nebuliser through the 0.22 µm bacterial retentive filter (Millex GV®)



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



## Storage Instructions

- Dilutions A through J (16 mg/mL through 0.031 mg/mL) should be stored at 36° to 45°F (2° to 8°C) in a refrigerator for no more than 2 weeks
- Unreconstituted powder should be stored at 59° to 86 °F (15° to 30°C)
- Freezing does not affect the stability of dilutions



## 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 Diluent (Shake well!)	OBTAIN DILUTION	VIAL NAME	COMPLETED
Provocholine® 100 mg	6.25 mL	16 mg/mL	VIAL A - 16 mg/mL	
3 mL from vial A	3 mL	8 mg/mL	VIAL B - 8 mg/mL	
3 mL from vial B	3 mL	4 mg/mL	VIAL C - 4 mg/mL	
3 mL from vial C	3 mL	2 mg/mL	VIAL D - 2 mg/mL	
3 mL from vial D	3 mL	1 mg/mL	VIAL E - 1 mg/mL	
3 mL from vial E	3 mL	0.5 mg/mL	VIAL F - 0.5 mg/mL	
3 mL from vial F	3 mL	0.25 mg/mL	VIAL G - 0.25 mg/mL	
3 mL from vial G	3 mL	0.125 mg/mL	VIAL H - 0.125 mg/mL	
3 mL from vial H	3 mL	0.0625 mg/mL	VIAL I - 0.0625 mg/mL	
3 mL from vial I	3 mL	0.031 mg/mL	VIAL J - 0.031 mg/mL	

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## 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 cover 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 6.25 mL of Diluent and insert into the Provocholine® 100mg vial. SHAKE WELL. This produces 6.25 mL of Provocholine® 16mg/mL, also known as **VIAL A – 16 MG/ML**.
6. Using the Provocholine® needle and syringe, remove 3 mL from VIAL A and transfer to the vial labelled VIAL B – 8 MG/ML. Using the Diluent needle and syringe, add 3 mL Diluent. Shake well. **THIS IS VIAL B – 8 MG/ML**.
7. Using the Provocholine® needle and syringe, remove 3 mL from VIAL B and transfer to the vial labelled VIAL C – 4 MG/ML. Using the Diluent needle and syringe, add 3 mL of Diluent. Shake well. **THIS IS VIAL C – 4 MG/ML**.
8. Using the Provocholine® needle and syringe, remove 3 mL from VIAL C, and transfer to the vial labelled VIAL D – 2 MG/ML. Using the Diluent needle and syringe, add 3 mL of Diluent. Shake well. **THIS IS VIAL D – 2 MG/ML**.
9. Using the Provocholine® needle and syringe, remove 3 mL from VIAL D – 2 MG/ML and transfer to the vial labelled VIAL E – 1 MG/ML. Using the Diluent needle and syringe, add 3 mL of Diluent. Shake well. **THIS IS VIAL E – 1 MG/ML**.



## Summary of Directions, continued

### Procedure continued:

10. Using the Provocholine® needle and syringe, remove 3 mL from VIAL E – 1 MG/ML and transfer to the vial labelled VIAL F – 0.5 MG/ML. Using the Diluent needle and syringe, add 3 mL of Diluent. Shake well. **THIS IS VIAL F – 0.5 MG/ML**.
11. Using the Provocholine® needle and syringe, remove 3 mL from VIAL F – 0.5 MG/ML and transfer to the vial labelled VIAL G – 0.25 MG/ML. Using the Diluent needle and syringe, add 3 mL of Diluent. Shake well. **THIS IS VIAL G – 0.25 MG/ML**.
12. Using the Provocholine® needle and syringe, remove 3 mL from VIAL G – 0.25 MG/ML and transfer to the vial labelled VIAL H – 0.125 MG/ML. Using the Diluent needle and syringe, add 3 mL of Diluent. Shake well. **THIS IS VIAL H – 0.125 MG/ML**.
13. Using the Provocholine® needle and syringe, remove 3 mL from VIAL H – 0.5 MG/ML and transfer to the vial labelled VIAL I – 0.25 MG/ML. Using the Diluent needle and syringe, add 3 mL of Diluent. Shake well. **THIS IS VIAL I – 0.0625 MG/ML**.
14. Using the Provocholine® needle and syringe, remove 3 mL from VIAL I – 0.5 MG/ML and transfer to the vial labelled VIAL J – 0.25 MG/ML. Using the Diluent needle and syringe, add 3 mL of Diluent. Shake well. **THIS IS VIAL J – 0.031 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.

