Start-Up Manual for the RaPID Assay® System

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INTRODUCTION

This manual is intended to act as a guide for first time users of the RaPID Assay® kits and the associated equipment and as a reference for experienced users. It contains information on how to set-up the required equipment and run Strategic Diagnostics Inc.'s (SDI's) RaPID Assays. For more detailed explanation, refer to the operating manual for individual pieces of equipment and to the package insert for each assay kit.

Before running the first assay, read thoroughly those sections referring to each piece of equipment to be used (Sections 1 - 8). Next proceed to Sections 9 and 10 to run the assay. Section 11 is provided to assist the operator in resolving problems which might be encountered.

If any of the material contained in this manual is unclear or if problems are encountered, please feel free to call SDI's Technical Support at (800) 544-8881.

SECTION 1 - RPA-I™ ANALYZER

The RPA-I Analyzer is a laboratory benchtop-based, single wavelength, dual beam, microprocessor-controlled analyzer. It can read the absorbances of calibrators and samples, perform mathematical computations, and report raw absorbances and sample concentrations with statistics. For a complete and detailed description of the RPA-I, please refer to the *RPA-I RaPID Analyzer Operations Manual* (SDI Part No. A00046).

ENIIIRONMENT

- 5° C to 33° C
- 10% to 85% humidity
- Flat, level surface away from strong sources of electromagnetic interference.
- No direct sunlight or drafts.
- Removed from sources of direct heat and moisture.
- Ventilation space at least 6 inches on sides and back.

UNPACKING AND INSTALLATION

1. Inspect the carton for visible signs of damage and note the condition of the SHOCK-WATCH indicator on the side of the carton. If damage has occurred, or a part is missing, immediately contact SDI.

2. Open the carton and remove the brown rectangular box from the gray packing material. (Save all boxes.) This box contains the power transformer, roll of paper, and Program Cartridge. Refer to Figure 1 for identification of shipping carton contents.

3. Lift off the gray packing material to reveal the photometer. Remove it from the carton.

4. Insert the Program Cartridge (with the white label facing up) into the Program Cartridge Holder found on the rear panel of the instrument. Push in until the white label is no longer visible (Refer to Figure 2).

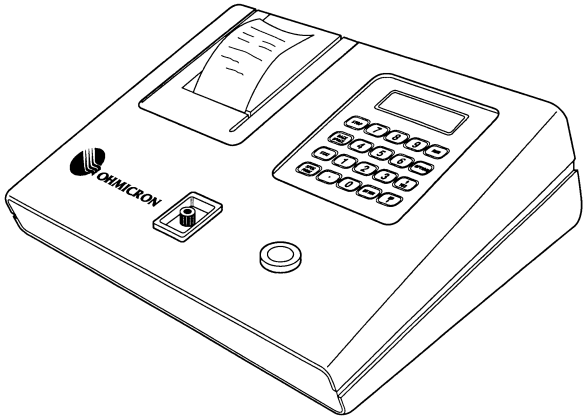
5. With the power **OFF** to the instrument, (bottom of the white toggle power switch should be depressed) insert the round end of the Power Transformer (notched end facing up) into the AC Power Connector found on the rear panel of the instrument. Plug the square end of the power cable into a grounded AC outlet.

6. The instrument is then activated by depressing the top of the white toggle power switch. The instrument will perform a "Self Test." During this short test, the various electronic components of the RPA-I are automatically analyzed. This includes checks of EPROM and RAM memory. If there are any abnormalities in these areas, the RPA-I will alert the operator with an "ERROR" message. If all the parameters are satisfactory, the "Select Command" prompt will appear and the operator may continue.

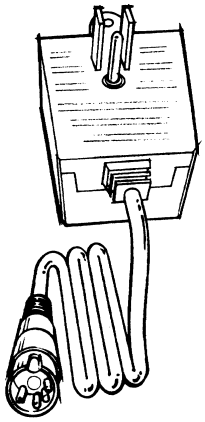
SHIPPING CARTON CONTENTS

The shipping carton should contain the following items:

RPA-I Analyzer with a 450/600nm filter block.

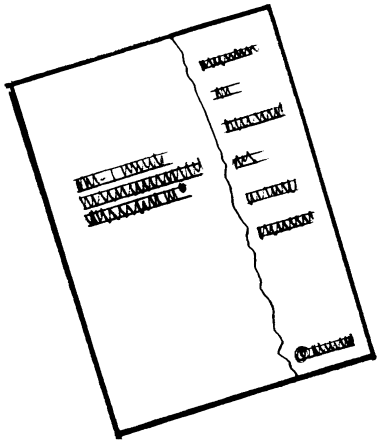


Domestic Power Cord/Mains Transformer

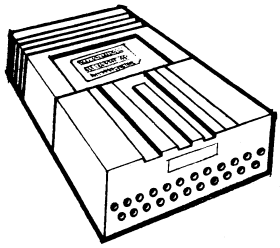


Error! Not a valid filename.

RPA-I Analyzer Operator's Manual



Program Cartridge



Printer Paper

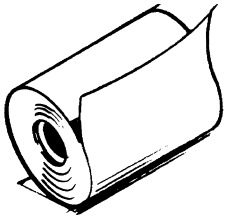


Figure 1. Shipping Carton Contents

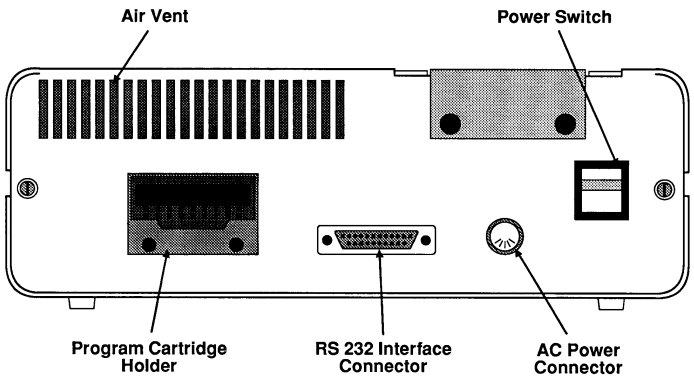


Figure 2. Rear Panel

SHORT OPERATING PROCEDURE FOR THE RPA-I

ALLOW THE RPA-I TO WARM UP FOR 30 MINUTES PRIOR TO USE. Avoid analyzing samples with air bubbles, foam, scratches, or foreign matter. The RPA-I performs a self test first. If all parameters are satisfactory, the "Select Command" prompt will appear. If there are abnormalities, an "Error" message will appear.
The RPA-I reports all results on a thermal paper printout. The unit is turned off by switching the power switch in the rear of the unit to the off position.

INSTRUMENT DISPLAY	OPERATOR RESPONSE
SELECT COMMAND	Press RUN
RUN PROTOCOL: Aldicarb, Atrazine, Alachlor, etc.	Scroll using the YES [š] or NO [›] until the desired protocol appears. Press ENTER.
SPL. REPLICATES: (1-5)	Press 1 (Press 2 if analyzing samples in duplicate, etc.). Press ENTER.
BLANK TUBE INSERT TUBE EVALUATING TUBE	Insert tube with 1 mL of washing solution
REMOVE TUBE (Beep)	Remove tube
CAL. #1 REP. #1 INSERT TUBE EVALUATING TUBE	Insert first standard replicate (0 ppb calibrator/tube #1).
REMOVE TUBE (Beep)	Remove Tube
Follow the prompts on the instrument display.	Note: Tube order is important here. The RPA-I expects to see the standards/calibrators in ascending order in duplicate, starting with 0 ppb.

After all the standards (calibrators) have been evaluated, the instrument will display:

PRINTING DATA LISTING XFORM DATA	Data will print.
PRINTING CURVE	Curve will print only if programmed to print (See Section 3 Special Functions - Instrument Functions: Print Curve).
CTRL. #1 REP. #1 INSERT TUBE EVALUATING TUBE	Insert Control Tube.
REMOVE TUBE (Beep)	Remove Tube.
EDIT CALIBRATORS YES/NO	Press NO if it is not necessary to edit the calibrators, press YES to edit (See Section 3 Run).
SPL. #1 REP. #1 INSERT TUBE EVALUATING TUBE	Insert first Sample Tube.
REMOVE TUBE (Beep)	Remove Tube.

Follow the prompts on the instrument display. After all the samples have been evaluated, press STOP.

EXPLANATION OF DATA

Bolded areas are explained in the right hand column.

```
Ø4-12-91  12:36:38

******** SDI ********

PROTOCOL :  ATRAZINE

TECH ID :_________________
LOT #   :_________________
EXP DATE:_________________

Data Reduct:Lin.Regression
Xformation:        Ln/LgtBÀ
Read Mode  :    Absorbance
Wavelength :        450 nm
Units      :           PPB

EQUATION OF LINE :
------------------
Slope     =    -Ø.842     À
Intercept =    -Ø.1Ø6     À
Corr (r)  =    Ø.9929     À

Transformed Data :
----------------
   Conc       Abs
--------------------
  -2.3Ø     1.926          À
   Ø.ØØ    -Ø.334          À
   1.61    -1.327          À


Calibrator Data:
----------------

  Conc     Abs  %CV  Predic
          Diff        %Diff
--------------------------
  Ø.ØØ  1.Ø32
        1.Ø24
  Mean  1.Ø28  Ø.5

  Ø.1Ø  Ø.889          Ø.1ØÀ
       -Ø.ØØ2          -2.4À
        Ø.9Ø6          Ø.Ø8
       -Ø.Ø18         -22.7
  Mean  Ø.897  1.4     Ø.Ø9À
       -Ø.Ø11         -11.8

  1.ØØ  Ø.442           1.23
        Ø.234          18.9
        Ø.416           1.39
        Ø.393          28.2
  Mean  Ø.429  4.2      1.31À
        Ø.311          23.7

  5.ØØ  Ø.228           3.91
       -1.Ø94         -28.Ø
        Ø.2Ø3           4.67
       -Ø.328          -7.Ø
  Mean  Ø.216  8.4      4.26
       -Ø.736         -17.3

--------------------------
```

Data Reduction

Method of transformation for data. Example, Ln refers to the natural log of the concentration and LgtB refers to the logit function of the absorbance divided by the absorbance at zero concentration.

Equation of Line

These values are the coefficients which describe a "best fit" or linear regression straight line where Logit (B/B_O) = slope x Log_e (conc. in ppb) + intercept. The Corr(r) is the correlation coefficient which indicates "goodness of fit" of the data to the best fit line. The square of this value represents the proportion of variance (on the y axis) that is explained by the linear regression.

Transformed Data

This section shows the average "transformed data" for each standard point. For example,
Log_e (0.1 ppb) = -2.30
Logit ($\frac{0.897}{1.028}$ or $\frac{B}{B_O}$) = 1.926

Calibrator Data

0.889 = observed absorbance
0.10 = observed concentration
-0.002 = known conc.(0.10) - observed conc.(0.10)*
-2.4 = concentration diff (-0.002) ' observed conc. (0.10) x 100*
1.4 = standard deviation of observed absorbances ' mean (0.897) x 100
4.2 = coefficient of variation (%CV) is calculated using absorbances

*For accuracy, the data reduction software of the RPA-I utilizes seven significant digit numbers although only three are displayed or printed.

```
Control Data :
--------------

Ctrl#   Abs    Conc
--------------------------
  1   Ø.274    2.93

ID:_____________________


Samples Data :
--------------

Spl#    Abs    Conc    %CV
--------------------------
  1   Ø.482    1.Ø2
      Ø.46Ø    1.13
Mean  Ø.471    1.Ø8    7.3À

ID:_____________________

  2   Ø.36Ø    1.84
      Ø.368    1.76
Mean  Ø.364    1.8Ø    3.Ø

ID:_____________________

  3   Ø.925    Ø.Ø7
      Ø.93Ø    Ø.Ø6
Mean  Ø.928    Ø.Ø6    4.7

ID:_____________________

  4   Ø.991    Ø.Ø2nd     À
      Ø.998    Ø.Ø1nd
Mean  Ø.995    Ø.Ø2nd17.7*À

ID:_____________________

  5   Ø.927    Ø.Ø6
      Ø.921    Ø.Ø7
Mean  Ø.924    Ø.Ø7    5.4

ID:_____________________

  6   Ø.23Ø    3.86
      Ø.233    3.78
Mean  Ø.232    3.82    1.4

ID:_____________________

  7   1.Ø38      nd       À
      1.Ø36      nd
Mean  1.Ø37      nd

ID:_____________________
END OF RUN
Ø4-12-91 12:39:24
```

Control Data

Displays absorbance and concentration of control sample. This concentration should be compared to the reported range located on the control vial label to assure the quality of the run.

Sample Data

7.3 = this %CV is calculated using the sample concentrations

"nd" indicates concentration below the "Normal Range Low" value entered during the protocol setup. This value is the least detectable dose (LDD) for RaPID Assay protocols.
"*" indicates the %CV exceeds the parameter setting limit.

An "nd" without a concentration indicates the absorbance measured is greater than the absorbance of the 0 ppb standard therefore a concentration cannot be calculated.

SECTION 2 - RPA-III™ ANALYZER

The RPA-III RaPID Analyzer is a hand-held microprocessor based unit with fully interchangeable filters for wavelength selection. The instrument features a liquid crystal display and is powered through a cable connected to an electrical outlet. By itself, the RPA-III does not provide a printout of results, nor does it perform mathematical functions. For a complete and detailed description of the RPA-III, please refer to the *RPA-III RaPID Analyzer Operations Manual* (SDI Part No. A00xxx).

UNPACKING AND INSTALLATION

1. Inspect the package for visible signs of damage and note the condition of the carton. If damage has occurred, or a part is missing, immediately contact SDI.
2. Open the carton and remove the photometer and power cord from the packing material.
3. Plug the square end of the power cord into a grounded 110 v AC outlet. Insert the other end into the back of the RPA-III.
4. The unit is turned off by unplugging the power cable from the unit.

SHORT OPERATING PROCEDURE FOR THE RPA-III

Before reading tubes, allow five minutes after powering the RPA-III for warm up. Avoid analyzing samples with air bubbles, foam, scratches, or foreign matter.

INSTRUMENT DISPLAY	OPERATOR RESPONSE
STANDARDIZE? Y/N	Press the **Zero/No** button.
ZERO BLANK	Insert the blank tube containing at least 1 mL of Washing Solution and press the **Zero/No** button.
READ	The RPA-III reads the blank tube and zeros the instrument (NOTE: for optimum performance, re-zero the unit after every ten readings. To re-zero, insert the blank and press the **Zero/No** button twice.)
00 0.000 ABS.	The unit displays the reading number and the absorbance. Remove tube.
READ SAMPLE	Insert the first tube and press the **Read/Yes** button.
01 X.XXX ABS.	The tube is read and the absorbance is displayed. RECORD THE ABSORBANCE VALUE.
02 X.XXX ABS.	Repeat for all standards, control, and samples.

CALCULATIONS

Using the graph paper provided with the RaPID Assay Kit, draw the standard curve by plotting the B/B_0 ratios versus concentrations using the absorbance data obtained above. Graph papers are specific for each method. Use only the graph paper supplied with each kit.

The mean absorbance value for the 0 standard is the B_0. The mean absorbance value for the other calibrators is the B value. Divide the absorbance of the standard, control or sample by the zero absorbance and multiply by 100 to obtain the % B/B_0. Draw the best straight line through all standard $\%B/B_0$ points ($\%B/B_0$ - y-axis, concentration - x-axis). Using the $\%B/B_0$ of the sample interpolate the concentration using the standard curve.

SECTION 3 - RPA-III™ FIELD KIT

The RPA-III RaPID Field Kit consists of the RPA-III Analyzer; a hand-held microprocessor based unit with liquid crystal display, and a portable case which contains a rechargeable battery, printer and tube cover. The Field kit also contains a power cord for battery charging and optional AC operation. The RPA-III Field Kit is intended for use outside of the laboratory environment.

For a complete and detailed description of the RPA-III, please refer to the *RPA-III RaPID Analyzer* Operations Manual (SDI Part No. A00xxx).

UNPACKING AND INSTALLATION

1. Seat the RPA-III firmly in the case making sure the battery/printer connection is inserted into the photometer.

2. Assuming the battery is charged, turn on both the printer and the RPA-III via the two power toggle switches.

Refer to the RPA-III Operating Manual for complete and detailed descriptions of operations.

SHORT OPERATING PROCEDURE FOR THE RPA-III FIELD SYSTEM

Allow the RPA-III to warm up for five minutes after switching it on before reading tubes. Avoid analyzing samples with air bubbles, foam, scratches, or foreign matter.

INSTRUMENT DISPLAY	OPERATOR RESPONSE
STANDARDIZE? Y/N	Press the **Zero/No** button.
ZERO BLANK	Insert the blank tube containing at least 1 mL of Washing Solution. Place the tube cover over the tube and seat it firmly on the reader. Press the **Zero/No** button.
READ	The RPA-III reads the blank tube and zeros the instrument (NOTE: for optimum performance, re-zero the unit after every ten readings. To re-zero, insert the blank and press the **Zero/No** button twice.)
00 0.000 ABS.	The unit displays the reading number and the absorbance. Remove tube.
READ SAMPLE	Insert the first tube. Place the tube cover over the tube. Press the **Read/Yes** button. The absorbance is displayed and printed.
01 X.XXX ABS.	The tube is read and the absorbance is displayed. RECORD THE ABSORBANCE VALUE.
02 X.XXX ABS.	Insert the next tube. Place the tube cover over the tube and press the **Read/Yes** button. The absorbance is displayed and printed.

CALCULATIONS

Refer to the appropriate *RPA-III Field Protocol* found in the *RPA-III RaPID Analyzer* Operations Manual for directions on *Calculations and Interpretation of Results* for qualitative or semi-quantitative results.

SECTION 4 - OTHER SPECTROPHOTOMETERS

REQUIREMENTS

This section describes the requirements for use of RaPID Assays with tube readers other than the RPA series photometers. These requirements are:

Absorbance Range: 0 - 2.0 AU

Wavelength: 450 nm.

Detection volume: 1 mL in cuvettes.

Drift: 9 0.005 AU per hour at 0 AU.

Linearity: 9 0.005 A or 2% difference from calculated regression line. Correlation coefficient,
r = 0.9995 or better.

Results reporting: to a display or printer.

Sipper cell: the operator must first establish pump cycle times that allow for 1 mL to be drawn up from the tube and provide delivery to the flow cell to be read. Once this has been established, a return cycle of two times the pick-up volume should be returned to avoid cross-over contamination.

Throughput: ability to read the tubes within *15 minutes* after the addition of stop solution to the assay tube.

CALCULATIONS

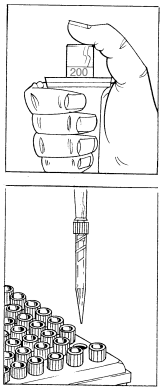
Using the graph paper provided with the RaPID Assay Kit, draw the standard curve by plotting the B/B_0 ratios versus concentrations using the absorbance data obtained above. Graph papers are specific for each method. Use only the graph paper supplied with each kit.

The mean absorbance value for the 0 standard is the B_0. The mean absorbance value for the other calibrators is the B value. Divide the absorbance of the standard, control or sample by the zero absorbance and multiply by 100 to obtain the % B/B_0. Draw the best straight line through all standard $\%B/B_0$ points ($\%B/B_0$ - y-axis, concentration - x-axis). Using the $\%B/B_0$ of the sample interpolate the concentration using the standard curve.

SECTION 5 - FIXED VOLUME PIPETTES

ASSEMBLY

Secure a tip on the pipette nose by pressing the nose cone **firmly** into a tip contained in the pipette tip rack.



USE

General

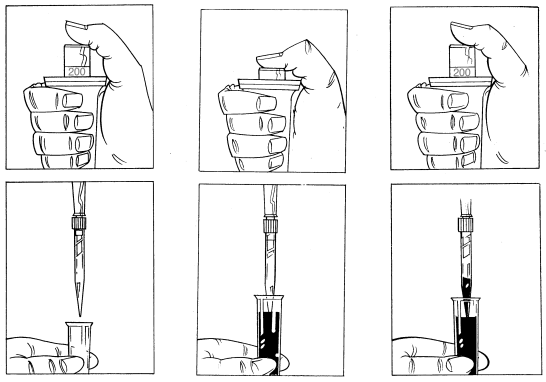
The piston stroke is divided into three sections: measuring, blow-out, and tip ejection.

First stop: required volume is defined and dispensed

Second stop: dispense any residual liquid

Third stop: tip ejection

Practice several pipette transfers with water using the following procedure:



1 2 3&4

Filling

1. Keep pipette almost vertical.

2. Press button down to the first stop. Immerse tip 2-3 mm into the liquid.

3. **Slowly** allow the button to glide back (never let it snap back).

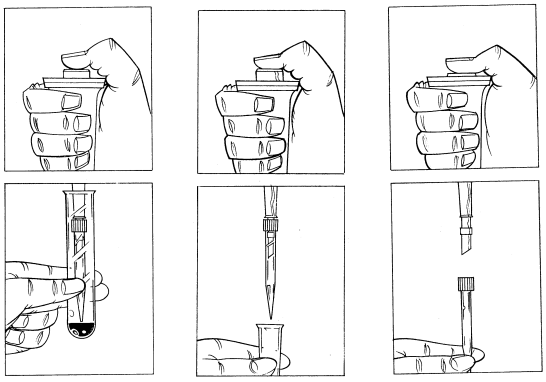
4. Slide tip out along the inside of the vessel.

Dispensing

5. Insert pipette almost to the bottom of the test tube. Touch the tip to the side of the test tube about 5 mm above the dispensed liquid. Press button down to the **second stop**.

6. Remove pipette from the test tube.

7. Press button down to the third stop and discard the tip.



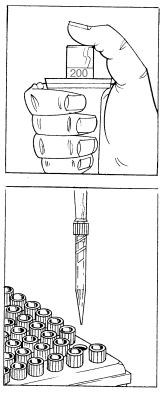
5 6 7

IMPORTANT: A new tip should be used for <u>each</u> standard or sample.

SECTION 6 - TRI-VOLUME PIPETTES

ASSEMBLY

Secure a tip on the pipette nose by pressing the nose cone **firmly** into a tip contained in the pipette tip rack.



USE

The tri-volume pipette will deliver the volume displayed opposite the red mark.

To select the volume to be delivered, press the button down to the horizontal mark (middle of the numbers) in the volume ring (e.g. 100--200--250) and turn to the right or left. The button must be returned to the fully extended position (numbers visible) to lock the required volume into place.

General

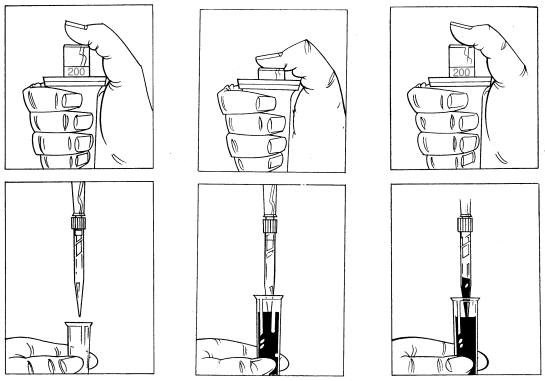
The piston stroke is divided into three sections: measuring, blow-out, and tip ejection.

First stop: required volume is defined and dispensed.

Second stop: dispense any residual liquid.

Third stop: tip ejection

Practice several pipette transfers with water using the following procedure:



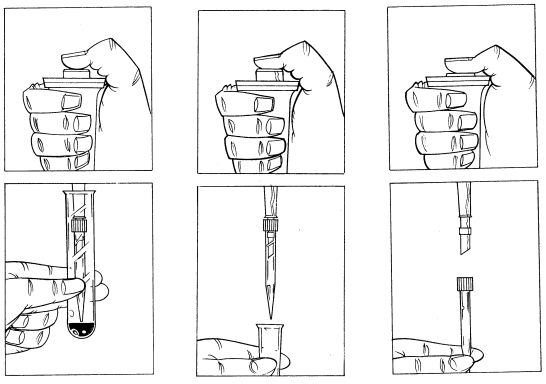
1 2 3&4

Filling

1. Keep pipette almost vertical.
2. Press button down to the first stop. Immerse tip 2-3 mm into the liquid.
3. **Slowly** allow the button to glide back (never let it snap back).
4. Slide tip out along the inside of the vessel.

Dispensing

5. Insert pipette almost to the bottom of the test tube. Press button down to the **second stop**.
6. Remove pipette from the test tube.
7. Press button down to the third stop and discard the tip.



5 6 7

IMPORTANT: A new tip should be used for <u>each</u> standard or sample.

SECTION 7 - REPEATER PIPETTES

ASSEMBLY

DESIGN PRINCIPLE:

1. Volume Selection Dial: To determine the pipette volume, the dial setting (1-5) is multiplied by the minimum pipette volume of the reservoir. See example under Reservoir/Use of the Repeater Pipette.

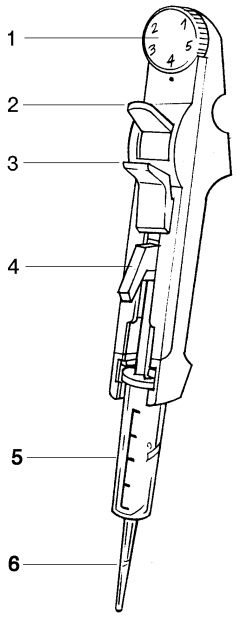
2. Pipette Lever: To deliver the selected volume, press this lever down until it stops.

3. Filling Lever: To fill the reservoir, slide this lever upward.

4. Locking Clamp: The locking clamp serves to firmly clamp the reservoir into the pipette.

5. Reservoir

6. Reservoir Cone



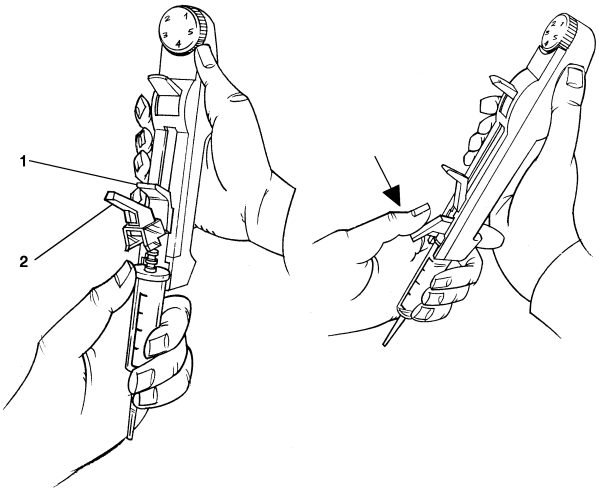
INSERTING A RESERVOIR:

1. Slide the filling lever (1) down until it stops.

2. Raise the locking clamp (2) upward.

3. Insert the reservoir <u>until it clicks</u> into position. Be sure the reservoir plunger is fully inserted into the barrel and the filling lever is completely down before attaching the reservoir to the pipette.

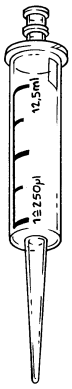
4. Lower the locking clamp to secure the reservoir in place.



USE

RESERVOIR:

The minimum pipetting volume and the maximum filling capacity are shown on each reservoir. The volume to be pipetted is obtained by multiplying the set number located on the volume selection dial by the minimum pipetting volume of the reservoir.



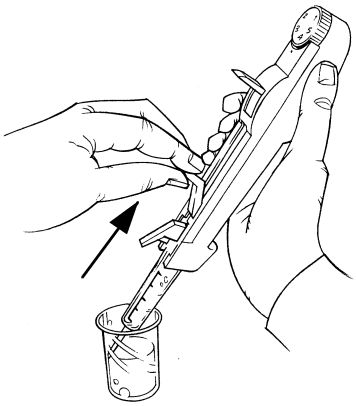
Example: 12.5 mL Reservoir:
Maximum fill volume = 12.5 mL
Minimum pipetting volume, Dial set to 1 = 250 µL
At dial setting 4: 4 x 250 µL = 1000 µL
Pipetted volume: 1000 µL

Practice several pipette transfers with water using the following procedure:

FILLING:

1. Immerse the reservoir cone into the liquid.

2. Fill by **slowly** sliding the filling lever upward.



3. Discard the first pipetting step by completely depressing the pipetting lever with a smooth, continuous motion until it stops.

4. Allow the pipetting lever to return to its starting position.

The repeater pipette is now ready for operation.

ADDITIONAL FILLING PRECAUTIONS:

Sliding the filling lever too quickly can cause excessive vacuum. This can cause tiny air bubbles to accumulate in the liquid which may lead to pipetting inaccuracies. If this occurs, empty and refill the reservoir.

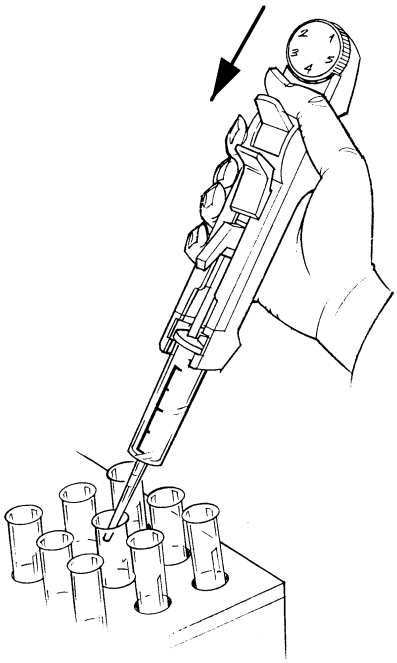
It is important to discard the first pipetting step in order to release any residual pressure from the pipetting system after filling and to prepare the system for precise pipetting.

Small bubbles in the reservoir beneath the piston do not affect pipetting accuracy due to an incorporated residual stroke lock which prevents the pipetting of any residual material after the last dispensing.

It is not necessary to completely fill the reservoir. Partially filling reservoirs does not affect pipetting accuracy.

PIPETTING:

A set of five labeled 12.5 mL (1=250 µL) reservoirs is needed for each RaPID Assay. They should be labeled with the assay name and the following: "conjugate 250 µL", "particles 500 µL", "wash 1 mL", "color 500 µL", and "stop 500 µL".



1. Check the volume selection dial for the appropriate setting.
   Enzyme conjugate Á set dial at 1
   Magnetic particles Á set dial at 2
   Wash solution Á set dial at 4
   Color reagent Á set dial at 2
   Stop reagent Á set dial at 2

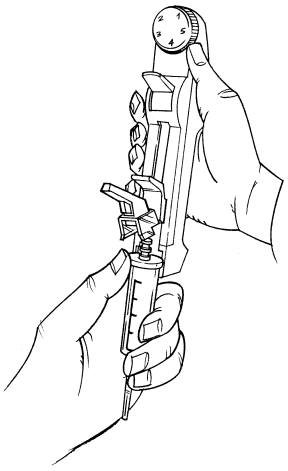
2. Hold the repeater at an angle so the tip is about one half inch below the tube rim without touching the rim or tube wall with the reservoir cone and aim the cone to deliver the liquid down the inside wall of the test tube.

3. Dispense the liquid by completely depressing the pipetting lever with a smooth, continuous motion until it stops.

4. Allow the pipetting lever to return to its starting position and repeat delivery into the next tube.

5. After the pipetting is completed, return the unused reagent to its container by holding the repeater unit over the container and pressing the filling lever down until it stops.

6. Prior to storage of the reservoirs, rinse the dedicated reservoirs twice with distilled water (keeping the reservoir in the pipette) by filling with 12.5 mL of distilled water each time and discarding the contents into a sink. Store syringes assembled (plunger inserted into barrel). Keep washed assembled syringes separated from each other. (Hint: An empty tray from the fixed volume pipette tips makes a handy storage rack). Reservoirs should be changed periodically (after 5-10 uses) since precision deteriorates with use.



REMOVING THE RESERVOIR:

Once the filling lever (1) is completely down, raise the locking clamp (2) upward and remove the reservoir.

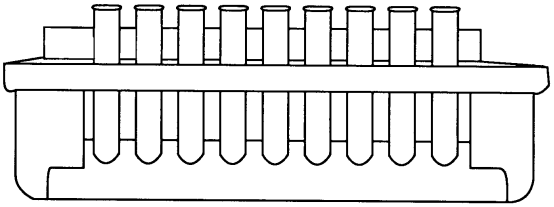
SECTION 8 - MAGNETIC RACK

USE

The magnetic rack is composed of two parts: the top rack that firmly holds the test tubes in place and the bottom base which contains the magnets.

ASSEMBLY

Place the rack on top of the base making sure they fit together securely and the test tubes are pushed down as far as they will go into the base.



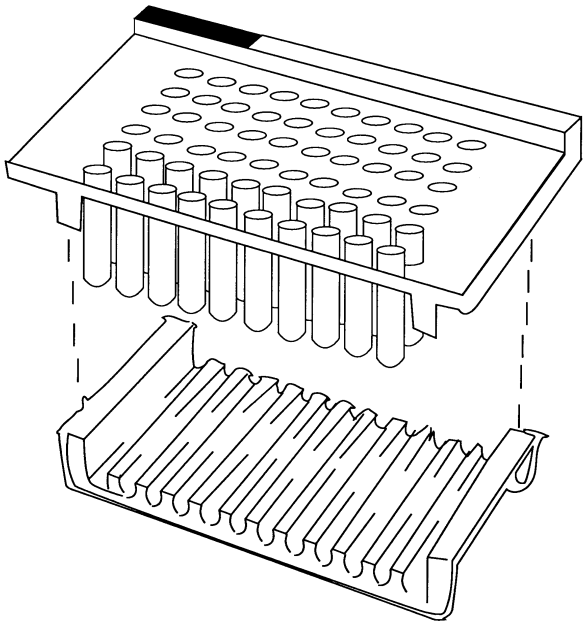
Assembled View

USE OF THE ASSEMBLED RACK

For separation steps (washing and decanting) - to pull the magnetic particles to the sides of the tubes allowing the unbound components to be discarded.

DISASSEMBLY

Separate the top rack from the bottom base.



Disassembled View

USE OF THE DISASSEMBLED RACK

For incubation steps - to allow the magnetic particles to remain suspended throughout the solution.

CARE

Do not submerge base in water. Clean with warm water and mild detergents.

CAUTION: Exposure to extreme heat or organic solvents could effect product's performance. Do not under any circumstances attempt to disassemble base to observe magnets. Magnets will be violently attracted to each other.

SECTION 9 - START-UP

MATERIALS NECESSARY TO PERFORM A RAPID ASSAY

1. RaPID Assay test kit with assay protocol, flowchart and package insert.
2. RPA-I, RPA-III with operating instructions (or equivalent spectrophotometer capable of reading 450 nm in a 1 mL sample size).
3. Magnetic rack separation unit.
4. Precision pipettes and appropriate tips, capable of delivering 100 µL, 200 µL, or 250 µL depending on method.
5. Repeating pipette, with appropriate reservoirs, capable of delivering 250 µL, 500 µL, and 1000 µL.
6. Thermolyne Maxi Mix™, Vortex Genie™ (or equivalent vortex mixer).
7. Blotting paper for decanting steps (usually use a stack of 5 kitchen type paper towels).
8. Permanent marking pens for labeling tubes.
9. Instructional video.
10. Laboratory timer or wrist watch.

ASSAY TIPS

Gently blot. Avoid **banging** the rack during the decanting steps.

Decant with the magnetic base joined to the top rack.

Wait a full two minutes for each separation step.

Bring samples and reagents fully to room temperature.

Vortex when specified without foaming.

Pay attention to the number of washes (2) and the amount of wash solution added (1 mL).

Add color reagent without the magnetic base joined to the top rack.

Remove as much liquid as possible from the test tubes when decanting.

Assure precise pipetting techniques.

Swirl the magnetic particles, without foaming, prior to addition.

PREPARATION AND RUNNING

Prior to performing your first RaPID Assay please do the following:

1. Read the entire package insert (found inside the box of reagents).

2. View the videotape (if available).

3. Gather together all the materials necessary to perform a RaPID Assay in a work space.

4. Remove the RaPID Assay kits from the refrigerator.

5. Remove the bottles from the kit box and place them on a countertop.

6. Allow the reagent bottles to come to room temperature.

7. Turn on the RPA-I or other spectrophotometer. The RPA-I should be warmed-up for 30 minutes.

8. Practice using the fixed volume and repeater pipettes with distilled water.

9. Label the top portion of the test tubes, with a permanent marker, in the following manner starting with the standard curve (in duplicate) followed by a control tube and sample tubes (in singlet).

Tube#	Contents of Tube
1,2	Zero Standard
3,4	Standard 1
5,6	Standard 2
7,8	Standard 3
9	Control
10	Sample #1
11	Sample #2
12	Etc.

10. Label 5 - 12.5 mL (1 = 250ml) reservoirs, with a permanent marker, with the following:
    the first reservoir = conjugate 250µL
    the second reservoir = particles 500µL
    the third reservoir = wash 1ml
    the fourth reservoir = color 500µL
    the fifth reservoir = stop 500µL

    In addition, add the name of the pesticide you are testing for to each syringe.

11. Continue with the **Assay Procedure** section of the package insert or flowchart.

Expected Results for RaPID Assays

- %CVs between standard duplicates of 10% or less.
- Absorbance readings for the 0 ppb standard greater than or equal to 0.800 for all assays.
- Corr (r) of 0.990 or greater for all assays.

SECTION 10 - ASSAY WORKSHEET

PURPOSE OF WORKSHEET

To aid in your evaluation of the assay kit, this familiarization run (a typical calibration curve plus proficiency samples) should be performed. Operators should repeat this run until the %CV of absorbances are consistently better than 10%. Experienced operators will obtain %CV's approaching 5% or better. The concentration values obtained on the control and the proficiency samples should be within the stated ranges.

FAMILIARIZATION RUN

Include in the run each standard in duplicate, a single control, and each proficiency sample in duplicate. Following the instructions in the kit package insert and found elsewhere in this manual, perform the assay.

RESULTS

RPA-I:

1. Observe the absorbance, the absorbance %CV, and the predicted result for each standard from the RPA-I printout (see Section 1 for a description of the printout).
2. Calculate the mean, SD, and %CV for the results from the standards and proficiency samples using a statistical calculator or the formulas given below the worksheet. Record in the following worksheet. Note that the %CV on the results may be significantly different than the absorbance %CV.
3. Compare to the above guidelines.

RPA-III or other photometers:

1. Record the absorbance value for each standard or sample in the worksheet which follows.
2. Calculate the mean, standard deviation (SD), and %CV on the absorbances for each standard and sample using a statistical calculator or the formulas given below the worksheet.
3. Using the graph paper provided with the kit, prepare a standard curve as described in the "Results" section of the kit package insert under "Manual Calculations". Read all samples and calibrators as individual points from the standard curve. Use the worksheet which follows to record the results.
4. Calculate and record the mean, SD, and %CV for the results using a statistical calculator or the formulas given below the worksheet.
5. Compare to the above guidelines.

INTERPRETATION

If you run two curves and do not achieve acceptable %CV's on the absorbances call SDI Technical Support (1-800-544-8881) to discuss your results.

NAME: _____

DATE: _____

METHOD: _____

LOT NUMBER: _____

EXPIRATION DATE: _____

Tube #	Contents	Absorbance	%CV of Absorbance	Result [ppb]	% CV of Result
1	0 ppb Standard				
2	0 ppb Standard				
3	Standard #1				
4	Standard #1				
5	Standard #2				
6	Standard #2				
7	Standard #3				
8	Standard #3				
9	Control				
10	Sample A				
11	Sample A				
12	Sample B				
13	Sample B				
14	Sample C				
15	Sample C				

Calculations -

n = # of samples Mean: $\bar{x} = \Sigma x/n$

Standard Deviation (S.D.): = $\sqrt{(\Sigma(x - \bar{x})^2/(n - 1))}$

Percent Coefficient of Variance (%CV): = (S.D./ $\bar{x}$) X 100

SECTION 11 - TROUBLESHOOTING

Symptom	Cause	Corrective Action
Increased Absorbance	Long incubation.	Adhere to incubation times.
	Washed tubes only once.	Be sure to wash tubes twice.
	Washed tubes with less than 1 mL of Washing Solution.	Check pipette for delivery of 1 mL of Washing Solution.
	Warm reagents.	Bring reagents to room temperature.
Decreased Absorbance	Banging rack during decanting.	Gently blot tubes, don't bang.
	Decanting without top rack joined to magnetic base.	Join rack and base prior to decanting.
	Short incubation.	Adhere to incubation times.
	Did not wait 2 minutes between washings.	Allow particles to separate between washings.
	Cold reagents.	Bring reagents to room temperature.
	Did not vortex after addition of color reagent or particles.	Vortex tubes after the addition of color reagent and particles.
	Addition of color reagent while top rack is joined to magnetic base.	Separate rack from base before adding color reagent.
Higher than expected results	Presence of cross reactants, particulate matter, or other interferences in the sample.	For particulate matter, filter samples with a 0.2 µm filter and re-assay. For cross reactants and possible interfering substances, dilute sample and re-assay.
	Inaccurate standard curve.	Re-run standard curve.
	Expired reagents and/or kit.	Discard and replace with a fresh kit.
	Drift in sample results from beginning to end of run.	Add all reagents in a consistent manner to entire rack within one minute.
Lower than expected results	Standards contaminated with analyte.	Discard and replace with a fresh kit.
	Inaccurate standard curve.	Re-run standard curve.
	Expired kit.	Discard and replace with a fresh kit.

Symptom	Cause	Corrective Action
Increased % CV's	Banging rack during decanting.	Blot, don't bang.
	Decanting without top rack joined to magnetic base.	Join rack and base prior to decanting.
	Did not vortex after addition of color reagent or particles.	Vortex tubes after the addition of color reagent and particles.
	Addition of 2 mL of wash solution instead of 1 mL.	Check pipette for delivery of 1 mL of liquid.
	Forgot to wait 2 minutes for separation.	Allow particles to separate between washings.
	Excessive wash solution remaining in tubes after decanting (may appear as bubbles).	After decanting, while holding top rack and bottom base together, allow tubes to drain in an inverted position for a few minutes. Also obtain more absorbent toweling.
	Imprecise addition of reagents.	Replace reservoir. Pipettes may need maintenance.
	Neglecting to vortex during the wash steps in the PCB procedure.	In the PCB RaPID Assay after adding the Washing Solution to each tube, vortex each tube for 1-2 seconds.