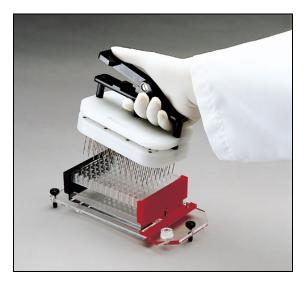
User Manual for the 96-Well Transfer Device Catalog #TRNDV96





Web site: www.onelambda.com

Advancing Transplant Diagnostics

Canoga Park, CA 91303-2801 Fax: (818) 702-6904 Revision 1 A color version of this document is available for viewing or printing at the One Lambda Web Site: www.onelambda.com/prod/micro/trandev/index.html

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Table of Contents

Getting Started 1
Unpacking the Device 1 Description of the Device 1 Product components 1 Accessories Included 1 Accessories Not Included 1 Safety 2
Using the Transfer Device
Washing the Device
Service7
Replacement Parts 7 Instructions for Return of the Device for Service 7 Replacing a Needle 7 Procedure for Replacing a Needle 7 Procedure for Cleaning Needles 11 Gasket Replacement 12 Procedure for Replacing a Gasket 12 Calibrating the Transfer Device 15 Transfer Device Calibration Procedure 15 Calibration Grid 16 Calibration Grid 18

Table of Figures

Figure 1: Transfer device stored in wash stand	1
Figure 2: Washing the needles	
Figure 3: Loading liquid	4
Figure 4: Format of Micro SSP™ Class I and II ABDR Tray 384 (SSPABDRX)	5
Figure 5: Replacement needles and screws are provided in a plastic tube	7
Figure 6: Transfer device placed in wash stand for removal of screws	8
Figure 7: Carefully remove needle from needle plate	8
Figure 8: Needle replacement tool with needle inserted notched end first	9
Figure 9: Detail of needle inserted in needle replacement tool notched end first	9
Figure 10: Seating a needle with the needle replacement tool and a mallet	9
Figure 11: Needle plate with all needles installed	10
Figure 12: Placement of six springs shown circled.	10
Figure 13: Illustration of Replacement Gasket	12
Figure 14: Needle plate in wash stand with all but 2 screws removed	13
Figure 15: Transfer device with plunger plate and seal plate removed and gasket	
exposed for inspection	13
Figure 16: Hex wrench in vertical position	14
Figure 17: Hex wrench in horizontal position	14
Figure 18: Screw replacement pattern for seal plate	14
Figure 19: Calibration screw on transfer device	15

Unpacking the Device

Carefully remove the 96-Well Transfer Device from the shipping box.

Important: Retain the shipping box and packing materials in case you need to return the unit to One Lambda for service.

Description of the Device

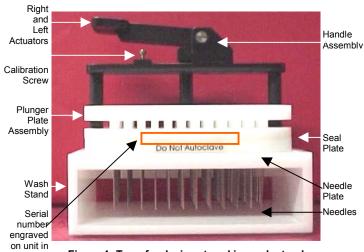


Figure 1: Transfer device stored in wash stand

Product components

Accessories Included

- 96-Well Transfer Device Stand (Catalog #TRNDVSTD)
- Needle Replacement Tool (Catalog #TRNDVNDTL)
- Maintenance Kit (Catalog #TRNDVMKIT) may be ordered separately for replacement parts

Accessories Not Included

- Transfer Device Alignment Plate (Catalog #TRNDVAP)—required for use with the Micro SSP™ Gel System
- Transfer Device Needle Replacement Kit (Catalog #TRNDVNKIT)—12 replacement needles

Revision: 1 TRNDV96_MAN.DOC

Page 1 of 18

Safety

The stainless steel needles used in this device may pose a puncture hazard. Keep hands and fingers clear of the needles to avoid breaking skin with the needles. When device is not in use, it should be placed in the wash stand to protect the needles.



- Designed for use with liquids; do not use with highly viscous liquids, volatile liquids, or oil.
 - Do not subject the gasket to liquid when it is exposed.
- Do not autoclave unit.
- Use only needle cleaning wires provided for this device.

Revision: 1 TRNDV96_MAN.DOC

Page 2 of 18

Using the Transfer Device

There are three key steps in use of the transfer device:

- 1. Washing the needles of the device.
- 2. Loading of reagent
- 3. Dispensing of reagent into 96 wells or tubes

Before using of the transfer device for the first time, wash the unit with water as described below.

Washing the Device

The wash step should be repeated at least five times (as outlined in the procedure below) to ensure that all reagent has been removed from the needles. It is very important to wash the unit thoroughly between patient samples or between reagents to avoid cross-contamination. Even a minute amount of DNA material left in the unit can compromise the results of a DNA typing.

Procedure for Washing the Unit



Figure 2: Washing the needles

- 1. Place a container of clean water (minimum of 3/8" deep) in wash stand and place on a stable work surface. (A pipette tip set box or cover may be used as the water container.)
- 2. Place transfer device in stand. Needles should be submerged.

Caution: Avoid jamming of needles in reservoir.

- 3. Press and release the **RIGHT** actuator at least five times to aspirate and dispense water. Remove from stand.
- 4. Press **RIGHT** actuator repeatedly to ensure water is expelled from the needles.
- 5. Dry needle tips by gently blotting them on a paper towel.

Revision: 1 TRNDV96_MAN.DOC

Page 3 of 18

Loading Liquid with the Device

Liquid is loaded from a plate or reservoir into the unit for transfer to a 96-well format gel or plate.

Procedure for Loading Liquid

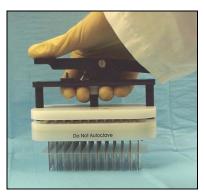


Figure 3: Loading liquid

Warning: Do not use the unit with highly viscous or volatile liquids.

- 1. Position unit over plate or reservoir from which liquid is to be loaded.
- 2. Press and hold LEFT actuator before touching needles to liquid.
- 3. Slowly release **LEFT** actuator to aspirate liquid into the needles.

Dispensing Liquid with the Device

Liquid is dispensed onto a 96-well format gel or plate.

Procedure for Dispensing Liquid

- For accurate placement of liquid on a One Lambda Gel System (Catalog # MGS-108) gel plate, we recommend using a Transfer Device Alignment Plate (Catalog # TRNDVAP). If you use the plate, align it over the gel prior to loading the transfer device.
- Bring transfer device, which has been loaded, to the gel or plate on which the liquid is to be dispensed. If you are using a transfer device alignment plate, the transfer device should mate with or meet the alignment plate to ensure correct placement on the gel. The needle plate should be flush with the alignment plate.
- 3. Press and hold the LEFT actuator to dispense samples onto the gel or plate.
- 4. While maintaining the **LEFT** actuator in the down position, move the transfer device away from the gel or plate.
- Verify that reagent has loaded properly on the gel or plate. If the reagent has not been loaded properly, return the transfer device to the gel or plate and dispense remaining liquid.
- 6. Complete steps in **WASHING THE UNIT** on page 3 before loading and dispensing a new reagent or sample.

Revision: 1 TRNDV96_MAN.DOC

Page 4 of 18

Using the Device with a 96-Well or 384-Well PCR Plate

One Lambda's 384-well Micro SSP[™] trays contain multiple tests. For example, the Micro SSP[™] ABDR HLA DNA Typing Tray (Catalog #SSPABDRX) contains 4 tests. Each test sample is loaded in a 96-well format. In the example shown in Figure 4, sample 1 is loaded in all of the white wells, sample 2 is loaded in all of the light gray wells, sample 3 is loaded in all of the dark gray wells, and sample 4 is loaded in all of the black wells.

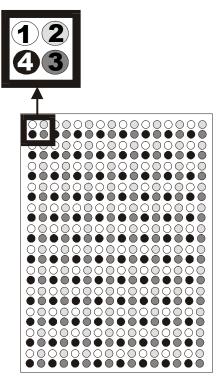


Figure 4: Format of Micro SSP™ Class I and II ABDR Tray 384 (SSPABDRX)

Procedure for Loading Samples from a 96-Well or 384-Well PCR Plate onto a 96-Well Gel

- Before transferring a sample to the gel, orient Transfer Device Alignment Plate (Catalog # TRNDVAP) over solidified gel.
- Wash Transfer Device 5 times with clean water by pressing and releasing RIGHT actuator repeatedly and then dry needles by gently blotting them on a paper towel and repeatedly pressing the RIGHT actuator to expel all water. (See Procedure for Washing the Unit on page 3.)
- For a 384-well tray, place post-PCR Well Identification Template (Catalog # 384TMP) on the plate. This assists you in transferring the correct sample onto the gel.

Revision: 1 TRNDV96_MAN.DOC

Page 5 of 18

- Press and hold the LEFT actuator of the transfer device before placing needles in tubes of the plate.
- While continuing to hold the LEFT actuator down, place needles in holes of template so that each needle tip touches the bottom of the tube.
- 6. Slowly release LEFT actuator to aspirate sample into the needles.
- 7. Bring transfer device (loaded with sample) to the alignment plate over the gel if you are using a Micro SSP™ Gel System.
- 8. Place needles into holes of the alignment plate to ensure correct placement of the sample on the gel.
- Press and hold LEFT actuator to dispense sample from needles onto gel. Do not release LEFT actuator.
- 10. While continuing to hold the **LEFT** actuator in the down position, move the transfer device and alignment plate away from the gel.
- Check gel to ensure that the sample has been loaded in all "wells" of the gel. (The gel is ready for electrophoresis.) If sample loading is not complete, return the alignment plate to the gel box and re-insert the transfer device. Continue to unload samples onto the gel.
 Note: Do not use more than 30 ml agarose gel in the Micro SSP™ Gel System. Too much gel may clog the needles of the transfer device. See Troubleshooting on page 16 if needles become clogged with agarose gel.
- 12. Be sure to complete Step 2 to wash the transfer device before using it to load samples from another PCR plate to a gel.
- 13. *For a 384-well*, repeat steps 1 through 11 until each sample has been loaded on a gel. (For example, for the SSPABDRX, which is a four-test tray, you repeat these steps four times—one time for each sample.)

Revision: 1 TRNDV96_MAN.DOC

Page 6 of 18

Replacement Parts

- The Maintenance Kit (Catalog #TRNDVMKIT) may be ordered so that the components may be used as replacement parts.
- Transfer Device Needle Replacement Kit (Catalog #TRNDVNKIT)—12 replacement needles

Instructions for Return of the Device for Service

Contact your One Lambda representative for instructions on returning the device for repairs or service. You will be asked to re-pack the unit in the original packing materials to ensure that the unit is properly protected for shipping.

Replacing a Needle

The transfer device is designed for replacement of needles. You should remove and replace any needle that is damaged to ensure correct operation of the device.

Procedure for Replacing a Needle

You will need the following items for needle replacement:

- Replacement needle(s) [for replacement needles, order the complete Maintenance Kit (Cat.#TRNDVMKIT) or the Needle Replacement Kit (Cat. TRNDVNKIT)]. See above.
- Needle replacement tool
- Pliers
- Hammer or mallet

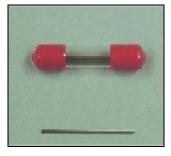


Figure 5: Replacement needles and screws are provided in a plastic tube.

Warning: Exercise care in handling of needles. Secure the needle plate before beginning the replacement process. Needles are very sharp and can present a puncture hazard.

Revision: 1 TRNDV96_MAN.DOC

Page 7 of 18

- 1. Place the transfer device upside down in the wash stand.
- 2. To remove the needle plate from the transfer device, use a 9/64" hex wrench to unscrew each of two screws at the ends of the needle plate. (See Figure 6.)

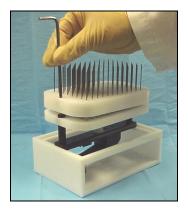


Figure 6: Transfer device placed in wash stand for removal of screws

3. To remove the needle to be replaced, grip the needle end firmly with pliers and pull with a slight twisting motion perpendicular to the needle plate. (See Figure 7.)

Caution: Do not apply bending motion to needle to avoid stress and possibility of needle breakage. If tension is applied in a direction perpendicular to the needle plate, the needle will come free.

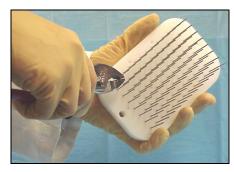


Figure 7: Carefully remove needle from needle plate

- 4. Note that the replacement needle has a small slit or notch at one end.
- 5. Place the replacement needle notched end first into the channel of the needle replacement tool. (See Figure 9.)

Caution: If needles are not placed notched end first into the plate, they will not operate correctly.

Revision: 1 TRNDV96_MAN.DOC

Page 8 of 18



Figure 8: Needle replacement tool with needle inserted notched end first

Figure 9: Detail of needle inserted in needle replacement tool notched end first

- 6. Using the needle replacement tool, place the end of the needle in the hole on the needle plate from which the old needle was removed.
- 7. Holding the needle tool perpendicular to the needle plate, carefully align the protruding end of the needle into the vacant position and push the needle perpendicular to the needle plate until the needle end of the replacement tool is flush with needle plate.
- 8. Sufficient hand force will seat the needle with the replacement tool. However, if you cannot seat the needle with hand force, use a hammer or mallet to tap on the end of the needle replacement tool until the tool is flush with the needle plate. (See Figure 10.) Multiple light taps are preferable to a single hard blow on the replacement tool.

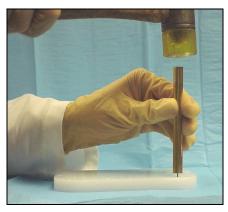


Figure 10: Seating a needle with the needle replacement tool and a mallet

- 9. Check each replaced needle for alignment by completing the following steps:
 - Sight down each row of needles along the length of the needle plate.
 - Sight down each row of needles along the width of the needle plate.

Revision: 1 TRNDV96_MAN.DOC

Page 9 of 18

If a needle is out of alignment with other needles in the row, apply a gentle bending hand force to that needle until it is straight and aligned with the other needles along both the length and width of the plate. (See Figure 11.)

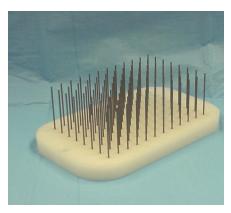


Figure 11: Needle plate with all needles installed

 Reassemble the unit. Place the needle plate on the handle assembly. Be sure to line the springs up in the mating holes. (See spring placement in Figure 12 below.) Fasten the needle plate to the base unit with the two screws you removed in step 2.

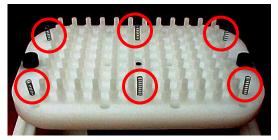


Figure 12: Placement of six springs shown circled.

Revision: 1 TRNDV96_MAN.DOC

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Page 10 of 18

Cleaning the Needles

Procedure for Cleaning Needles

- 1. Place transfer device upside down on wash stand or hold it upside down in your hands.
- Use a One Lambda Needle Cleaning Wire (Catalog #TRNDWKIT, Transfer Device Needle Cleaning Wire Kit – 12 replacement needle cleaning wires).
- 3. Slide the long end of the L-shaped wire into each needle and slide the wire in and out to remove any built-up residue or dirt.

Note: The wire is just slightly longer than the needle so that you cannot puncture the gasket with the wire. Do not depress the actuators while cleaning the needles.



- Do not use cleaning wires other than those provided by One Lambda for use with this product. Use of an incorrect wire may puncture the gasket, which will lead to failure of the unit.
- 4. Once the desired needles are clean, dispense and wash the transfer device to ensure that the transfer device is operating correctly.

Revision: 1 TRNDV96_MAN.DOC

Page 11 of 18

Gasket Replacement

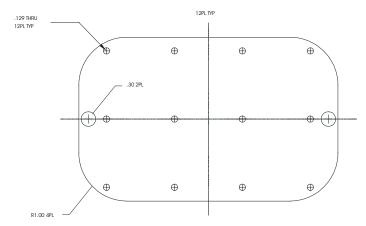


Figure 13: Illustration of Replacement Gasket

Procedure for Replacing a Gasket

The transfer device requires a flexible gasket (illustrated in Figure 13), which must be replaced when damaged. A puncture or tear in the gasket indicates the need for replacement. The unit must be disassembled to replace the gasket. For convenience, you may replace the gasket using the instructions supplied here. If you wish to have the gasket replaced by One Lambda engineering staff, contact your One Lambda Representative to arrange to have the gasket replaced.

- 1. Place the transfer device upside down in the wash stand.
- To remove the needle plate from the transfer device, use a 9/64" hex wrench to unscrew each of the two screws at the ends of the needle plate. (See Figure 6 on page 8.)
- 3. Keep handle assembly upside down for ease of re-assembly.
- 4. Pull needle plate free from handle assembly.

Warning: It may require some force to separate the seal plate from the needle plate. Exercise care in handling needle plate. Needles are sharp and can present a puncture hazard. Also, take care to avoid bending the needles.

- 5. Place needle plate in wash stand to stabilize it. (See Figure 14.)
- 6. Use a 3/32" hex (Allen) wrench to remove the 12 screws that secure the seal plate to the needle plate.

Revision: 1 TRNDV96_MAN.DOC

Page 12 of 18



Figure 14: Needle plate in wash stand with all but 2 screws removed

7. Lift off the seal plate and inspect the gasket. (See Figure 15.)



Figure 15: Transfer device with plunger plate and seal plate removed and gasket exposed for inspection

- 8. Remove the gasket.
- Clean the seal plate and needle plate contact surfaces thoroughly with water and dry with lint-free paper towels.

Caution: Do not scratch these surfaces or use abrasives on any sealing surface.

- 10. Make certain that all needles and wells are clean and free of debris. Clean needles with cleaning wire, if needed. (See **Procedure for Cleaning Needles** on page 11.)
- 11. Place new gasket on the clean dry surface of needle plate.
- 12. **Important:** Align the 12-hole pattern of the gasket with the pattern in the needle plate as precisely as possible.
- 13. With the gasket precisely aligned on the needle plate, place seal plate on the assembly with the top up.
- Place the unit so that the serial number on the front edge of the plate faces you while you are assembling the unit. (See Figure 1 for placement of the serial number.)
- 15. Use care to minimize horizontal motion while placing the seal plate to avoid moving and mis-aligning the gasket.
- 16. Replace screws in the seal plate using the following steps:

Caution: Make sure that gasket is clear of each screw hole. If a screw is allowed to catch on the gasket, the gasket may be torn or punctured.

Revision: 1 TRNDV96_MAN.DOC

Page 13 of 18

- a. Replace the screws in the 12 holes of the seal plate using the tightening pattern shown in Figure 18. Screws should turn easily if the gasket is properly aligned. This will ensure that the gasket is tightened uniformly between the plates.
- b. Tighten the screws in the pattern shown below, starting at screw one and tightening in order through screw 12, until all screw heads are flush with the seal plate.
- c. With the hex wrench in a vertical position, repeat the tightening pattern to tighten screws the maximum amount.



Figure 16: Hex wrench in vertical position

d. With the hex wrench in a horizontal position, repeat the tightening pattern one additional time.



Figure 17: Hex wrench in horizontal position

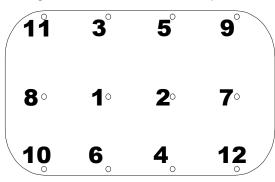


Figure 18: Screw replacement pattern for seal plate

17. Reassemble the unit. With handle assembly already upside down with the six springs in place, place the needle plate assembly on top of the upside down plunger plate assembly.

Revision: 1 TRNDV96_MAN.DOC

Page 14 of 18

Important: Be sure to line the springs up in the mating holes.

18. Fasten the needle plate to the base unit with the two screws you removed in step 2.

Calibrating the Transfer Device

We recommend that the transfer device be calibrated every one thousand trays to ensure that the appropriate amount of reagent is being dispensed. The purpose of calibration is to ensure that the average amount dispensed by the needles falls within a predefined range. The tolerance of the transfer device is $\pm 20\%$ of the target dispense volume of 12 µl (or 9.6 µl – 14.4 µl).

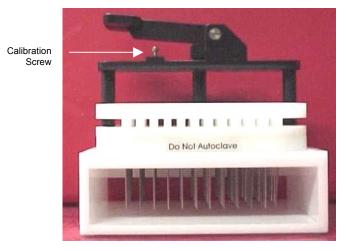


Figure 19: Calibration screw on transfer device

Transfer Device Calibration Procedure

You will need the following items to calibrate the transfer device:

- 80 μl calibration syringe (for example, Robbins Scientific Model #1060.00.0)
- Serum bag or hydrophobic surface
- Water reservoir
- Transfer device

The following steps are completed with the LEFT (dispense) actuator:

- 1. Depress the actuator and submerge needles in water reservoir. Release the actuator to absorb water. Then, depress the actuator again to dot a series of 96-wells on a dry, hydrophobic surface (such as a serum bag).
- Using a clean calibration syringe, aspirate one well at a time in each row (random column) to check if the volume is within the calibration range (9.6 μl – 14.4 μl).

Revision: 1 TRNDV96_MAN.DOC

Page 15 of 18

- 3. If the average volume is a value outside of the range $11.5 \,\mu$ l $12.5 \,\mu$ l, adjust the **LEFT** calibration screw to correct the volume (up to decrease and down to increase the volume).
- 4. Repeat step 3 until the average volume is within the specified tolerance $(11.5 \ \mu l 12.5 \ \mu l)$.
- To begin recording values on the Calibration Grid (page 17—make additional copies as needed), dot a new series of 96 wells. Sample and record only two rows at a time.
- Using a clean calibration syringe, aspirate one well at a time across the first row. Record the volume, starting at well 1H, 1G, 1F through 1A. Continue this process for the second row, 2H, 2G, 2F, and so forth, across the row.
- 7. Dot a new series of 96 wells, and repeat step 6 until all rows are complete.
- 8. When you have recorded information for each well, compute an average and record this value. Verify that the average volume for all wells is within the calibration range $(11.5 \ \mu l 12.5 \ \mu l)$.

The following steps are completed with the **RIGHT** (wash) actuator. It is not necessary to record values for the **RIGHT** actuator unless desired or required by your laboratory's protocol.

- 1. Depress the actuator and submerge needles in water reservoir. Release the actuator to absorb water. Then, depress the actuator again to dot a series of 96-wells on a dry, hydrophobic surface (such as a serum bag).
- 2. Using a clean calibration syringe, aspirate one well at a time in each row (random column) to check if the volume is within the calibration range (12.8 μ l 19.2 μ l).
- 3. If the average volume is a value outside of the range 16 μ l \pm 20%, adjust the **RIGHT** calibration screw to correct the volume (up to decrease and down to increase the volume).
- 4. Repeat step 3 until the volume is within the specified tolerance.

Troubleshooting

Before calling One Lambda for assistance with your transfer device, there are some simple steps you can take to resolve problems with the unit. Please refer to the following table for some common problems and their solutions.

Problem	Cause	Solution
Needle clogged with agarose gel	 Too much agarose 	 Do not use more than 30 ml of agarose gel in the gel box. To remove gel from needles, soak needles in warm water and let the gel run out of the needles. Then, proceed with normal needle cleaning procedure.
	 Actuator was released before needles were fully removed from alignment plate over the gel and agarose 	 To remove gel from needles, soak needles in warm water and let the gel run out of the needles. Then,

Revision: 1 TRNDV96_MAN.DOC

Page 16 of 18

	was aspirated into the needles	proceed with normal needle cleaning procedure.
Not enough reagent in well or on plate	Gasket or needle has exceeded useful life span	Replace gasket and/or needle.
Needle does not aspirate or dispense reagent	Needle placed upside down (un-notched end out) in plate.	Replace needle with notched end out.
Problem	Cause	Solution
No reagent in well or on plate	Gasket or needle has exceeded useful life span	Replace gasket and/or needle.
Reagent is dispensed unevenly in wells or on plate	 Gasket or needle has exceeded useful life span. 	 Replace gasket and/or needle.
	 Spring is missing or damaged. 	 Check for missing or damaged springs; replace springs as needed.
	 Needles do not align with wells 	 Make sure all needles are seated at the same height in the plate; make sure needle rows are aligned along both the width and length of the transfer device.
Needle leaks	 Leak in gasket Leak at the junction of the needle and the plate 	 Replace gasket. Replace needle; seal needle at base with super glue.
Transfer device sticks to alignment plate or does not slide easily out of alignment plate	 User is not maintaining needles perpendicular to the alignment plate when pulling the transfer device out of the alignment plate 	 Pull transfer device out of alignment plate in a vertical direction perpendicular to the alignment plate.
	There is gel on the needles.	 Do not use more than 30 ml of agarose gel in the gel box. To remove gel from needles, soak needles in warm water and let the gel run out of the needles. Then, proceed with normal needle cleaning procedure.
Transfer device actuator levers stick or become stiff.		 Loosen the two end top screws on the handle. Depress the actuators until smooth and tighten screws again.

Revision: 1 TRNDV96_MAN.DOC

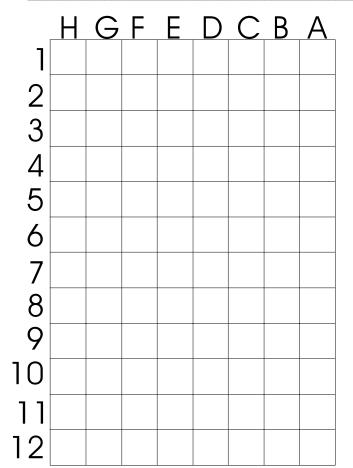
Page 17 of 18

Calibration Grid

Device Serial Number: _____

Date of calibration:

Technician:



Average amount dispensed _

Revision: 1 TRNDV96_MAN.DOC

Page 18 of 18