MANUAL FIXED or FLOATING PIN TOOL CLEANING AND LIQUID SAMPLE TRANSFER

OVERVIEW

There are several key steps in the successful use of pin tools:

1. ***Clean Pins.*** The first and most important step is to start with clean pins. It is strongly recommended that V&P's specially developed VP 110 Pin Cleaning Solution is used at the beginning and end of each day to keep the pins clean. For a list of V&P Scientific Pin Tool Cleaning Essentials and Accessories please see final page.

2. ***Blotting.*** Another key step is blotting onto a lint-free blotting material after delivery to the last recipient microplate and between wash solutions. Blotting greatly reduces carry-over. Blotting with lint-free blotting paper ensures that the pins do not pick up lint which will affect the volume transferred. V&P carries several different formats of Lint-Free Blotting Media:

3. ***Washing.*** Three wash solutions are recommended.

    a. The wash solution in the first reservoir will depend on the type of sample being transferred:

        i. For nucleic acid and biological (i.e., cell) transfers, use 10% bleach.

        ii. For peptide and protein transfers, use 0.1% SDS.

        iii. For small molecule compound transfers, use 50:50 DMSO:distilled H_2O or other solvent.

        iv. For transfers where particulate debris may accumulate, such as spotting onto agar, set up the first wash with a pin cleaning pad (VP 426 or VP 421S, see final page).

    b. The second solution can be distilled H_2O, or 100% alcohol if transferring small molecule compound libraries.

    c. Alcohol is recommended for the last wash solution due to its solvent characteristics and fast evaporation rate.

    d. NOTE: Always use distilled H_2O, not deionized H_2O in all pin tool applications. Long term exposure to deionized H_2O will damage the stainless-steel pins. V&P offers a variety of wash reservoirs: static, flowing, and disposable reservoirs.

4. ***Triple-Dip.*** When dipping the pins into source or recipient microplates and wash solutions, it is important to triple-dip into each solution. This mixes the liquid, loads and washes the pins to give more reproducible results. The pins should be completely removed from the liquid prior to each dipping to achieve maximum effect.

5. ***Withdrawal Speed***. The speed of pin withdrawal from the liquid during the final dip into the source microplate will determine the volume transferred. The speed of the pins during the first dipping (washing/mixing) steps can be fast. Please see the following webpage for more information:

PROTOCOLS

Pin Cleaning and Washing During Liquid Transfer with V&P Scientific Manual Pin Tools

I. Routine or Daily Cleaning
   - Before starting an experiment and after finishing an experiment.

II. Liquid Sample Transfer and Washing Between Transfers
   - Example protocol for liquid sample transfer from one microplate to another.
   - Recommended washing after sample transfer for prevention of sample cross-contamination during an experiment.

III. Heavy Duty Cleaning for Neglected Pin Tools
   - When pins have a buildup of material after extensive use without cleaning.

For a list of V&P Scientific Pin Tool Cleaning Essentials and Accessories please see final page.

Pin Cleaning and Washing During Liquid Transfer with V&P Scientific Manual Pin Tools

I. ROUTINE OR DAILY CLEANING:

Before starting an experiment *and* after finishing an experiment.

A. Set up three wash reservoirs: Use VP 420 Glass Alcohol Reservoirs or pipet tip box lids.

1. Fill wash reservoir #1 with 1:5 dilution of VP 110 Pin Cleaning Solution (please see Technote 40 for more information about VP 110).

2. Fill wash reservoir #2 with distilled H_2O.

3. Fill wash reservoir #3 with alcohol (ethanol, isopropanol, or methanol).

4. Adjust the level of wash solution in each reservoir as follows:

   a. The first reservoir should wash slightly higher on the pins than the "high water mark" which results from dipping into microplates during an experiment.

b. The second wash reservoir should be slightly higher than the first reservoir.

c. The third wash reservoir should be slightly higher than the second reservoir.

5. Next to each wash reservoir place a single piece of VP 522 Lint-Free Blotting Paper on top of a stack of paper towels. The paper towels should be stacked so that the layers are even across the area where the pin tool will be blotted.

B. Washing pins: It is critical for the tip of the pin to move up and down <u>through</u> the meniscus with each dip into the wash solutions.

1. Dip pins into a 1:5 dilution of VP 110 Pin Cleaning Solution 3 times through the meniscus. Blot pins on VP 522 Lint-Free Blotting Paper.

2. Repeat wash and blot with VP 110 Pin Cleaning Solution.

3. Dip into distilled H_2O wash, 3 times through the meniscus. Blot pins on VP 522 Lint-Free Blotting Paper.

4. Repeat wash with distilled H2O.

5. Dip into alcohol wash, 3 times through the meniscus. Blot pins on VP 522 Lint-Free Blotting Paper.

6. Repeat wash with alcohol.

C. Drying pins: Dry pins with portable hot-air dryer.

1. Hold pin tool in working position with pins pointing down.

2. Blow warm air, from below, up towards the pin tips.

    a. Move air from portable dryer across all the pin tips.

3. Gently turn pin tool upside-down with pins pointing up.

    a. All the floating pins should move through the float holes and rest on the top plate.

    b. Blow warm air, from the sides, between the float plates and top plate.

4. Turn pin tool back to working position with pins pointing down.

    a. If no pins remain stuck in the “up” position, then pin tool is ready to use.

    b. If a few pins move slowly or remain stuck in the “up” position, repeat blowing warm air from below and sides while gently rocking the pin tool upside-down to right-side up until all pins are moving freely.

II. LIQUID SAMPLE TRANSFER AND WASHING BETWEEN TRANSFERS:

A. Set up three wash reservoirs in VP 420 Plastic Wash reservoirs and/or VP 420 Glass Alcohol Reservoir:

1. Wash solution in reservoir #1 will depend on the sample being transferred:
    a. For nucleic acid and biological (cell) transfers, use 10% bleach.
    b. For peptide and protein transfers, use 0.1% SDS.
    c. For small molecule and compound transfers, use 50:50 DMSO:distilled H_2O.
    d. For transfers where particulate debris may accumulate, such as spotting onto agar, set up the first wash with a pin cleaning pad (VP 426 or VP 421S, see page 5).
2. Fill wash reservoir #2 with distilled H_2O (or 100% alcohol, if transferring compound libraries.).
3. Fill wash reservoir #3 with alcohol (ethanol, isopropanol, or methanol).
4. Adjust the level of wash solution in each reservoir as follows:
    a. The first reservoir should wash slightly higher on the pins than the "high water mark" which results from dipping into microplates during the experiment.
    b. The second wash reservoir should be slightly higher than the first reservoir.
    c. The third wash reservoir should be slightly higher than the second reservoir.
5. Next to each wash reservoir place a single piece of VP 522 Lint-Free Blotting Paper on top of a stack of paper towels. The paper towels should be stacked so that the layers are even across the area where the pin tool will be blotted.

B. Example of liquid sample transfer from source microplate to recipient microplate:

1. Dip pins into source plate:
    a. Dip pins into source plate to desired depth
    b. Lift the pin tool so the pins are just barely out of the source plate solution.
    c. Repeat dipping into source plate two more times.
    d. Raise pins out of source plate.

2. Transfer pins to plate:

    a. Dip pins into recipient plate to desired depth.

    b. Lift the pin tool so the pins are just barely out of the plate solution.

    c. Repeat dipping into plate two more times.

    d. Raise pins out of plate.

    e. Blot the pins on the VP 522 Lint-Free Blotting Paper so the pins float up ~2mm.

    f. Let the pins dwell on the Lint-Free Blotting Paper for 2 seconds.

C. Washing after each source-to- plate transfer:

1. Dip pins into first wash reservoir.

    a. It is critical that during each dip the tip of the pin is moved up and down through the meniscus.

    b. Move pins up and down through the meniscus 3 times.

    c. Blot pins on VP 522 Lint-Free Blotting Paper.

2. Dip pins into second wash reservoir.

    a. Move pins up and down through the meniscus 3 times.

    b. Blot pins on VP 522 Lint-Free Blotting Paper.

3. Dip pins into third wash reservoir.

    a. Move pins up and down through the meniscus 3 times.

    b. Blot pins on VP 522 Lint-Free Blotting Paper.

4. Allow pins to air-dry or use a portable hot-air dryer as described below.

D. Drying pins: Dry pins with portable hot-air dryer.

1. Hold pin tool in working position with pins pointing down.

2. Blow warm air, from below, up towards the pin tips.

    a. Move air from portable dryer across all the pin tips.

3. Gently turn pin tool upside-down with pins pointing up.
    a. All the floating pins should move through the float holes and rest on the top plate.
    b. Blow warm air, from the sides, between the float plates and top plate.
4. Turn pin tool back to working position with pins pointing down.
    a. If no pins remain stuck in the "up" position, then pin tool is ready to use.
    b. If a few pins move slowly or remain stuck in the "up" position, repeat blowing warm air from below and sides while gently rocking the pin tool upside-down to right-side up until all pins are moving freely.

III. HEAVY DUTY CLEANING FOR NEGLECTED PIN TOOLS

A. Clean pins in 65°C Ultrasonic Cleaning Bath.

1. Use 1:100 dilution of Micro90 Ultrasonic Cleaning Solution in an ultrasonic bath.
2. Immerse the lower 9 mm of pins in bath for 1 minute.
3. Rinse pin tips under hot, running tap water for 30 seconds.
4. Rinse pin tips with distilled H_2O squirt bottle.
    a. If liquid has splashed on float plate, rinse float plate with distilled H_20 squirt bottle.
5. Rinse pin tips with alcohol squirt bottle.
    a. If liquid has splashed on float plate, rinse float plate with alcohol squirt bottle.
6. Blot excess liquid with VP 522 Lint-Free Blotting Paper that is on top of a stack of paper towels. The paper towels should be stacked so that the layers are even across the area where the pin tool will be blotted.
7. Dry pin tool briefly with portable hot-air dryer or continue with "Cleaning with Pin Cleaning Solution" below.

B. Drying steps: Dry pins with portable hot-air dryer.

1. Hold pin tool in working position with pins pointing down.
2. Blow warm air, from below, up towards the pin tips.
    a. Move air from portable dryer across all the pin tips.

3. Gently turn pin tool upside-down with pins pointing up.

    a. All of the floating pins should move through the float holes and rest on the top plate.

    b. Blow warm air, from the sides, between the float plates and top plate.

4. Turn pin tool back to working position with pins pointing down.

    a. If no pins remain stuck in the "up" position, then pin tool is ready to use.

    b. If a few pins move slowly or remain stuck in the "up" position, repeat blowing warm air from below and sides while gently rocking the pin tool upside-down to right-side up until all pins are moving freely.

C. Clean pins in 1:3 dilution of VP 110 Pin Cleaning Solution.

1. Set up wash reservoirs as in Routine/Daily Cleaning (page 2) except that the VP 110 is diluted 1:3. Also set up an additional VP 522 Lint-Free Blotting Paper with a stack of paper towels.

2. Immerse the lower 9 mm of pins in VP 110. Move pins up and down through the meniscus 3 times.

3. Blot on VP 522 Lint-Free Blotting Paper.

    a. Check wet spots on Blotting Paper for uniformity.

    b. If spots are not uniform in size, continue to dip and blot a few more times until spots are uniform.

4. If spots are still not uniform after multiple dips and blots, slot pins may have dust or debris in the slots.

    a. Rinse the pin tips under hot, running tap water.

    b. Scrub the pin tips with VP 425 Pin Cleaning Brush or VP 426 Pin Tip Cleaning Pad.

    c. Rinse again with hot, running tap water.

    d. Repeat Step 3.

    e. If necessary, examine pins under a dissecting microscope to check for lint or debris on the pins or in the slots.

5. Dip pins into VP 110 again and blot to check spot size.

6. Once spot sizes are uniform, dip again in VP 110 by moving up and down through the meniscus 3 times.

7. Immerse the lower 9 mm of pins in VP 110. Soak pins in VP 110 for 30 minutes.

8. Rinse pin tips under hot, running tap water briefly.

9. Blot the pins briefly on piece of Lint-Free Blotting.

10. Dip pins into first wash reservoir.

    a. Move pins up and down through the meniscus 3 times.

    b. Blot pins on VP 522 Lint-Free Blotting Paper.

11. Dip pins into second wash reservoir.

    a. Move pins up and down through the meniscus 3 times.

    b. Blot pins on VP 522 Lint-Free Blotting Paper.

12. Dip pins into third wash reservoir.

    a. Move pins up and down through the meniscus 3 times.

    b. Blot pins on VP 522 Lint-Free Blotting Paper.

13. Dry pins with portable hot-air dryer as described above.

V&P PIN CLEANINIG ESSENTIALS:

- **VP 110 Concentrated Pin Cleaning Solution-** Used to clean the pin tips, removing any build-up or residue. Provided as a 5X concentrate

- **VP 522 Lint-Free Blotting Paper-** Used to blot replicator pins in between liquid transfers and washes. This is recommended because blotting on standard paper towels can leave lint particles in on the pin tips, interfering with the loading and unloading of liquid on the pin.

- **VP 425 Pin Cleaning Brush-** Used before and after an experiment, keeps the standard-length replicator pins free of particulate debris. 2.4 cm nylon bristles.

- **VP 425A Pin Cleaning Brush-** Used before and after an experiment, keeps the long length replicator pins free of particulate debris. 8.5 cm nylon bristles.

- **VP 426 Pin Cleaning Pad-** Used either before and after or during an experiment, keeps the tips of replicator pins free of particulate debris. Useful in applications where cells are being transferred to agar or membrane. 4 mm nylon bristles.

V&P PIN CLEANING ACCESSORIES:

- **VP 421 Plastic Wash Reservoir-** Used in pin washing steps to hold wash reagents such as 10% bleach, buffers, DMSO or water. For alcohol washes we recommend VP 420 below. Case of 5.

- **VP 421S Pin Cleaning Pad/Wash Reservoir Combo-** Used in in pin washing steps where it is important scrub off any particulate debris such as cells or agar.

- **VP 420 Glass Alcohol Reservoir-** Used in the sterilization of the replicators by flaming. Some labs use plastic reservoirs, but if flaming alcohol drops into them, the plastic burns, dumping flaming alcohol all over the lab bench. This covered dish is useful for storing the alcohol as well as VP110 Pin Cleaning Solution (above) for reuse.

- **VP 540DB Heavy Duty Blotting System-** The key to this Blotting Station is a super absorbent polypropylene pad that will hold up to 27ml of fluid before it is saturated. The pad is placed in an Omni Tray (VP 413) with a single piece of VP 540D Lint-Free Paper on top. The blotted liquid is transferred from the Lint-Free Paper to the pad and a barrier holds it in the pad. This super absorbent pad allows for Blotting Stations to last longer, just replace the VP 540D Lint-Free Paper on top as needed.

- **VP 904 Pin Dryer-** Used to speed the drying time of the pins and decrease assay cycle time.

- **VP 565A Reagent Dispenser-** Used to maintain a constant volume in the wash reservoirs by regulating the flow of wash fluid through a VP 549H2O or VP 549ETOH Spillway Reservoir.