TECHNICAL NOTE 10

TIPS ON THE CARE AND USE OF MULTI-BLOT™ REPLICATORS AND REGISTRATION ACCESSORIES

Disinfecting:

The Replicators can be sterilized by hot air oven, autoclaving, treating in 10% bleach or alcohol flaming. The pins can be cleaned between source plates by dipping briefly in a 10% bleach solution, followed by a series of two sterile dH2O baths (all in tip lid boxes), then a 99% isopropanol bath in a non flammable VP 420 glass reservoir. The pins can be air dried, using a hair drier or by flaming the isopropanol. Between baths, remove the liquid from the pin tips by blotting on a lint-free blotting paper (VP 522) as lint from paper towels will interfere with uniform liquid transfers. This blotting step is also very important to reduce carry over.

If you flame, do not blot the isopropanol before igniting and be prepared for a "whoosh" upon ignition. Keep the isopropanol reservoir and blotting paper at least 3 feet from the flame. We strongly advise you to use a non-flammable alcohol reservoir such as the VP 420 Pyrex® alcohol reservoir to avoid laboratory fires.

If you don't flame, blot on the lint-free blotting paper and let the isopropanol evaporate. The evaporation can be speeded up with a hair dryer. It is important that the pins be dry before going into the next source plate.

** Flaming just entails igniting the alcohol on the pins and NOT heating the pins directly in a Bunsen burner which can damage the corrosion resistant properties of the stainless steel pins.

Care:

Before each day’s use we recommend that the pins be cleaned with VP 110 Pin Cleaning Solution, which is designed to clean stainless steel and condition the pins. If the pins should be coated with organic material they can be mechanically cleaned with the VP 425 brush and Ivory dish detergent. If you have access to an ultrasonic bath, we recommend using MICRO 90®, from Cole Parmer®, at a 1/100 dilution in the ultrasonic bath. If you use an ultrasonic bath, hold the Replicator in the bath without letting the pins touch the bottom of the reservoir (the vibrating bottom surface of the sonicator's reservoir may damage the pin tips). The Cole Parmer Catalog #P-08857-02 Ultrasonic Cleaner is ideal for cleaning the Replicators. It is not necessary to clean the replicators in an ultrasonic cleaner if you clean the pins with bleach and brush with detergent after each day's use.

Use: (Liquid-to-Liquid and Liquid-to-Membrane transfers)

1. Place a LIBRARY COPIER™ (VP 381, VP 381C, VP 381D, VP 381F, VP 381M or VP 381N) over a 48, 96, 384 or 1536 well source plate with the single alignment hole side of
the device closest to the last row of the plate. Slide the LIBRARY COPIER™ to make sure the plate is seated within the device and therefore registered.

2. Place a second LIBRARY COPIER™ (VP 381, VP 381C, VP 381D, VP 381F, VP 381M or VP 381N) over a 48, 96, 384 or 1536 well reception plate with the single alignment hole side of the device closest to the last row of the plate. Slide the LIBRARY COPIER™ to make sure the plate is centered and seated within the device and therefore registered.

3. Hold a sterile 48, 96, 384 or 1536 MULTI-BLOT™ Replicator at a 45° angle to the source plate LIBRARY COPIER™ and 20° angle to the left alignment hole. Place the right guide pin into the right alignment hole. Slowly decrease the 20° angle and place the left guide pin into the left alignment hole. Rotate the Replicator forward until guide pins line up vertically and slide down the alignment holes and the Replicator pins drop into the wells (see diagram).

4. Hold the LIBRARY COPIER™ in one hand and mix contents of wells by raising and lowering the Replicator 3X through the meniscus with the other hand. The speed at which the pins are removed from the wells on the final withdrawal will affect the size of the hanging drops and the amount of liquid on the sides of the pin. Removing the pins quickly from the source plate produces large, hanging drops on the tips of the pins and more liquid on the sides. We recommend removing the pins on the final withdrawal at a slow even speed each time (~.5 cm/sec). This action produces very uniform transfers from plate to plate and reduces the amount of liquid hanging on the tip and sides of the pins. Performing this operation with the LIBRARY COPIER keeps the pins in the middle of the well and prevents hanging drops from being accidentally touched off.

5. To deliver to another microplate with liquid in the wells, dip and raise the pins 3 times through the recipient plate's meniscus. Blot the pins on lint free paper to reduce carry over if the pin tool is put back into the source plate or to the wash bath.

6. Repeat steps 3-5 for each replicate plate as needed.

7. To deliver to a membrane, have a soft absorbing pad under the membrane (VP 522, VP 521, or VP 521V) and press gently on the replicator. We recommend using one of our MULTI-PRINT Registration systems (VP 382, VP 382B, VP 382D or the FU-MEEI registration system) to prevent the pins from making skid marks on the membrane.

8. Repeat steps 3, 4 and 7 for each replicate blot as needed.

Test your Replicator using dye (5% red food coloring) in 10 mM Tris, pH 8.0 with 0.005% Sarcosyl or Tween 20 as wetting agents in water.
Note: If you are having problems with varying volumes of liquid on the tips, clean the pins with the VP 110 Pin Cleaning Solution. Cleaning with the V&P Pin Cleaning Solution will reduce the surface tension on the pin and this will solve 99% of your pin loading problems. Also, you can add 0.005% Sarcosyl, Tween 20, protein or carrier DNA to lower the liquid surface tension.

WARNING:

- Do not soak in bleach solutions for a long period of time as this can corrode the stainless steel pins.
- Do not soak in deionized water as this can corrode the stainless steel pins.
- Do not heat the pins directly in the Bunsen Burner flame.

The VP 110 pin cleaning solution contains a dilute acid solution which can strip the protective anodized surface off the anodized aluminum replicator bases or float plates. If you accidentally get the VP 110 pin cleaning solution on an anodized surface, quickly rinse it off with water.

If a replicator should be accidentally dropped and the pins be bent, they can be straightened. Sight down the pin rows and place the barrel of a retracted ball point pen over the bent pin and gently push until the pin lines up with both pin rows and pin columns.

Registration Accessories:

Sterilization:

The LIBRARY COPIERS™ (VP 381, VP 381A, VP 381B, VP 381C, VP 381D, VP 381F, VP 381M and VP 381N) and the COLONY COPIERS™ (VP 380 and VP 380D) do not contact sterile surfaces and therefore do not need to be sterilized. They are cleaned by wiping with 80% isopropanol. Both of these accessories are made of unstress-relieved polypropylene and cannot be autoclaved.

The MULTI-PRINTs™ (VP 382, VP 382B, VP 382D and VP 382F series) are made from polycarbonate and can be sterilized by autoclaving, or by dipping in a 10% bleach solution. Often sterilization is not required and the MULTI-PRINT™ is merely cleaned by wiping with 80% isopropanol. After multiple uses or multiple autoclavings you may have to replace the double sticky tape on the bottom of the MULTI-PRINT™. Replacement tape (VP 435) can be obtained from V&P Scientific, Inc. This is a special tape with a high tac surface to stick to the MULTI-PRINT and a low tac surface to stick to the membrane. The low tac surface allows the tape to release the membrane without tearing it.

Care:

The alignment holes on the registration accessories can be made more visible by tracing the beveled surface with a felt tip alcohol resistant marker from VWR.