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An inexpensive method for applying nitrogen evaporation to hexane-containing 24- or 96-well plates

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Abstract

A method is described for assembling an evaporation manifold from a cell culture flask, which allows for efficient nitrogen evaporation of hexane from 24- and 96-well plates. The precursor parts are readily available in most research laboratories. The nitrogen evaporation manifold is inexpensive, could possibly be used with other organic solvents, and appears ideal for a small number of samples in a multi-well format.

Introduction

The removal of a volatile organic solvent (hexane) from a lipid sample is often accomplished by subjecting the sample to a steady flow of nitrogen in the absence of oxygen (Komarek 1967; Ramirez-Zacarias et al. 1992). A traditional nitrogen evaporator consists of long metal probes arranged in a circle, suitable for evaporating solvents from test tubes. While adapting a staining protocol in test tubes for use in our laboratory (Ramirez-Zacarias et al. 1992), we needed to evaporate hexane from wells in 24- and 96-well cell culture plates. Subsequently, we devised an inexpensive (but effective) apparatus for delivering steady streams of nitrogen gas to multi-well plates. The procedures for the establishment and validation of evaporation for a 24-well format are described.

Materials

1. Plasticware
   a. Cell culture flask, 175 cm^2 – #353112, BD Falcon[1]
   b. 24-well plates- Cellstar®, No. 662 160, Greiner Bio-One[2]
   c. 96-well plates- Costar, #3595, Corning Inc.[3]
   d. 10 μl pipet tips- MBP®, #3500, Molecular BioProducts Inc.[4]

[1]BD Biosciences, Two Oak Park, Bedford, MA 01730.
[2]Greiner Bio-One, 1205 Sarah St., Longwood, FL 32750.
[3]Corning Inc., One Riverfront Plaza, Corning, NY 14831.
[4]Molecular BioProducts Inc., 9880 Mesa Rim Road, San Diego, CA 92121.

2. Miscellaneous supplies

   a. Needle – 16 gauge-Monoject # 200037, Sherwood Medical Company[5]
   b. Bunsen burner – #17928-027, VWR International[6]
   c. Hemostat-#25601-060, VWR International[6]
   d. Chalk
   e. Paper
   f. Cellophane tape
   g. Support ring – #60120-102, VWR International[6]
   h. Support stand – #60110-200, VWR International[6]
   i. Clamp – #21570-126, VWR International[6]
   j. Tubing – Nalgene # 8007, Nalge Nunc International[7]
   k. Rubber stopper – size 6, #59580-229, VWR International[6]
   l. Tubing connector (polypropylene) – 3 1/2 inch, tapered ends, VWR International[6]

3. Chemicals

   a. Super glue (cyanoacrylate)
   b. Hexane – #9309-01, JT Baker[8]
   c. Tank of nitrogen gas – Air Liquide America Corp.[9]

Methods

Constructing the apparatus

Common laboratory supplies were used to construct an evaporation manifold (Figure 1). The base consisted of a 175 cm^2 cell culture flask with the cap removed. A template was made from either a 24- or 96-well plate by applying colored chalk to the rims of the wells, placing a piece of white paper on the chalked well rims, and transferring the circular pattern of the wells to the paper by rubbing. The paper was then affixed to the surface of the flask with cellophane tape (Figure 2a). A hemostat was used to grasp a 16-gauge needle, which was then heated to a red glow in the flame of a Bunsen burner and used to bore (melt) a hole in the center of each circle through the paper and the plastic (Figure 2b). The paper template was then removed.

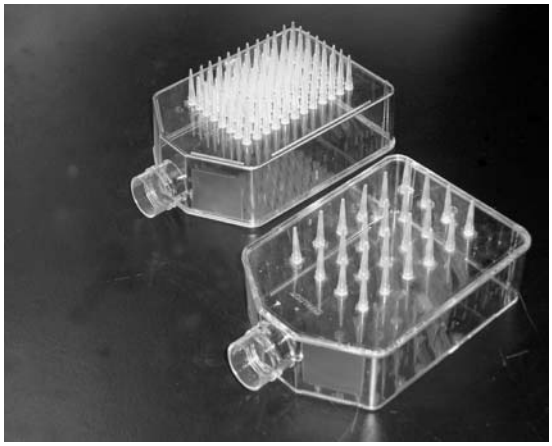

Figure 1. Evaporation manifold in either a 24- or 96 well format.

The nitrogen delivery nozzles were manufactured from 10 μl pipet tips whose ends were snipped with scissors to make a larger diameter opening (Figure 3a). The larger end of the tip was coated with a thin film of superglue (cyanoacrylate) (Figure 3b), and the tip was then pressed down over a hole in the flask (Figure 3c). This process was repeated for each tip and hole. Care was taken to ensure that the openings in all pipet tips were consistent. The apparatus was then allowed to dry for several hours to set the glue.

The total delivery system for nitrogen consisted of a nitrogen tank, tubing, a support stand, a three-prong clamp, a right-angle clamp, a # 6 rubber stopper, a polypropylene connector, and the flask/nozzle manifold (Figure 4a). A hole was bored through the rubber stopper to accommodate a 3.5 inch connector, the tubing was attached to the connector as it exited the stopper, and the manifold was held in place by two clamps attached to the support stand (Figure 4b). The nitrogen source was turned on for at least 5 min to allow the gas to equilibrate in the manifold. Next, the

[5]Sherwood Medical Company, St. Louis, MO 63103.

[6]VWR International, 1310 Goshen Parkway, West Chester, PA 19380.

[7]Nalge Nunc International, 75 Panorama Creek Drive, Rochester, NY 14625.

[8]Mallinckrodt Baker Inc., 222 Red School Lane, Phillipsburg, NJ 08865.

[9]Air Liquide America Corporation, Houston, TX 77056.

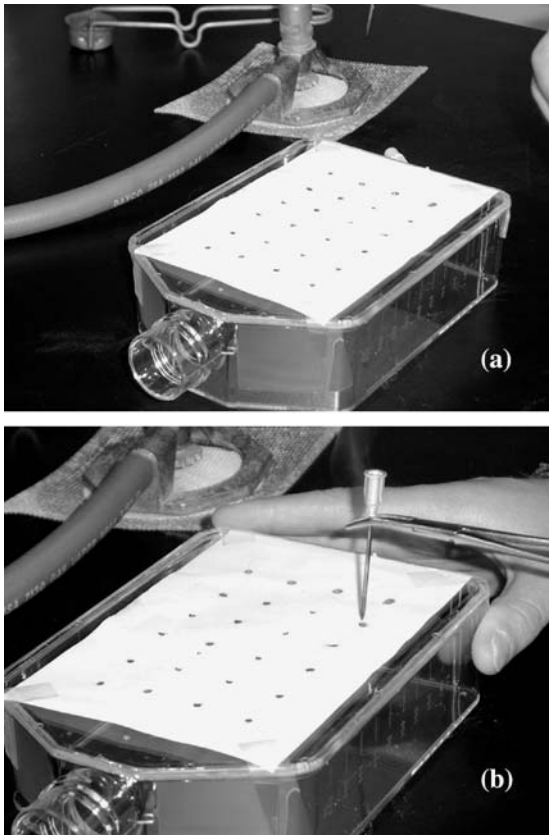

Figure 2. Creating the matrix of holes in the flask. (a) A paper template is affixed with tape to a 175 cm^2 cell culture flask. The black dots on the paper mark the centers of the well circles. (b) A heated disposable hypodermic needle is used to melt the holes through the plastic body of the flask.

whole apparatus was lowered over either a 24- or 96-well plate. The wells were then bathed with a steady stream of nitrogen gas until the solvent was evaporated (Figure 4c), usually about 10 min.

Determining evaporation times

The uniformity of evaporation of the hexane in the wells was a goal in the design of this manifold. To this end, an experiment was designed to determine the evaporation times of hexane in wells of a 24-well plate. The apparatus was set up in an unobstructed position in the center of a chemical fume hood, and its position was marked. A buffering zone (manifold equilibrium) was created by running a continuous flow of nitrogen (10 psi) through the manifold for 5 min before lowering it

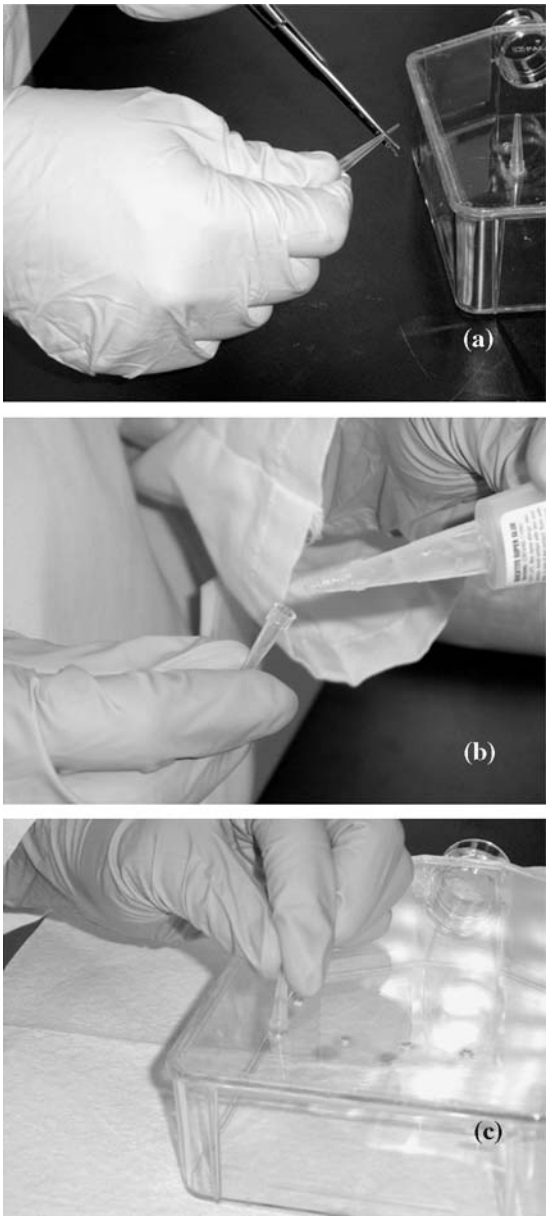

Figure 3. Affixing the tips to the flask. (a) A 10 μl pipette tip is snipped with scissors to increase the diameter of the opening. (b) A thin layer of superglue is applied to the larger opening of the tip. (c) The tip is centered over the hole in the plastic and pressed on to the surface of the flask.

over the plate. One ml of hexane was slowly pipetted into each well of a 24-well plate, and the plate was placed directly under the apparatus. Then the manifold was lowered over the plate in a level manner, and each well was checked to ensure that

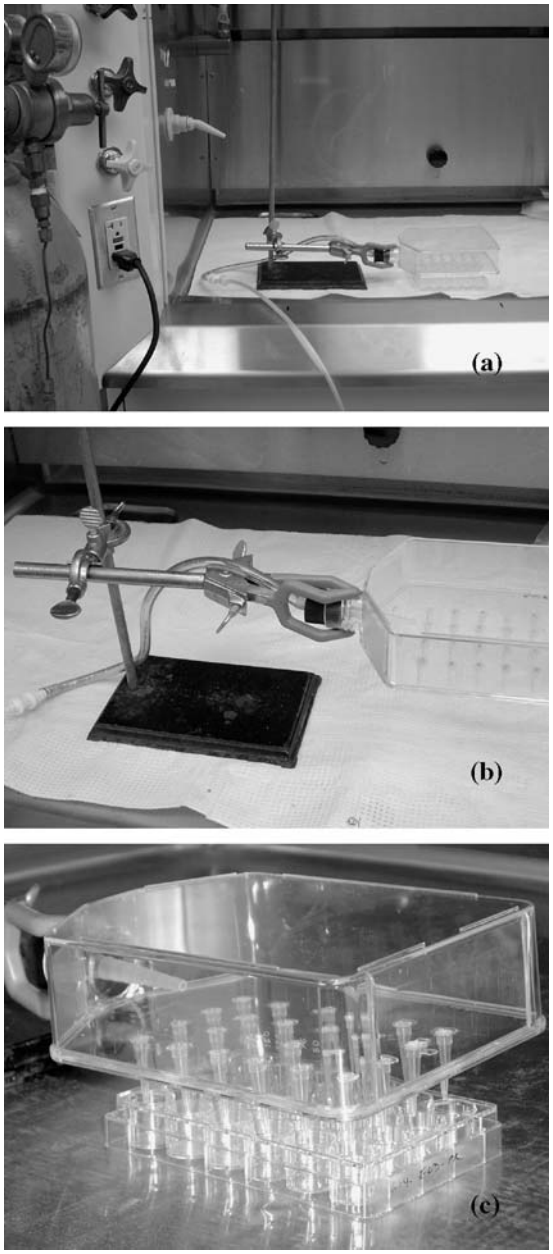

Figure 4. Set up for nitrogen evaporation. (a) A nitrogen gas tank is connected to the flask via tubing, a plastic connector and a rubber stopper. Two clamps affix the flask to the support stand. (b) The stand, clamps and flask manifold are prepared for evaporation. (c) The evaporation manifold (24-well format) is suspended over a 24-well plate.

the tips were centered and reaching about 1/3 of the way into each well. A timer was started, and the evaporation times of the wells were observed and recorded. Because it was physically impossible to follow all 24 wells at once, 12 wells per plate were selected for observation. However, at least one time during these experiments, every well in the 24-well plate was evaluated. All wells, in general, reacted similarly. To create a 'no buffering zone' (no manifold equilibrium), the manifold was lowered over the plate before the nitrogen gas was turned on.

Results and discussion

When adapting a method to quantitate oil red O in our laboratory experiments (Ramirez-Zacarias et al. 1992), we needed to devise an apparatus to evaporate a hexane solvent from lipid samples in multi-well plates. We were able to assemble this device from common items found in our cell culture laboratory. The resulting evaporation manifold is an effective but inexpensive way to deliver steady streams of nitrogen into individual wells of either a 24- or 96-well plate.

Evaporation time. There was no significant difference ($p > 0.05$) between the wells of plates in which the manifold was/was not equilibrated with nitrogen prior to being placed over the treatment culture plates (Figure 5). The data set for each treatment represents four replicate experiments of selected wells (12 wells per plate; $n = 48$) and their times of evaporation. Further, placing the apparatus in an unobstructed position in the center of the hood did not decrease the variation of evaporation. In all instances oxidization of the samples was at a minimum as long as nitrogen continuously entered the well.

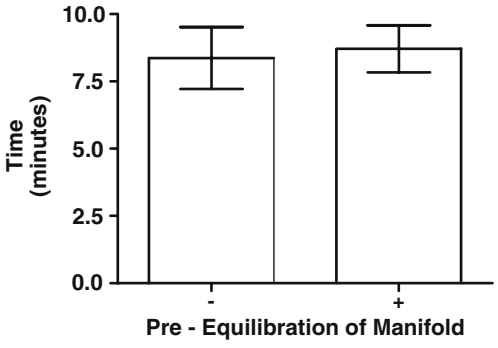

Figure 5. The evaporation times of hexane in a 24-well plate, with (+) and without (−) equilibration of the manifold. The data set for each column represents four replicate experiments (12 wells per plate; $n = 48$) and their times of evaporation.

Manifold assembly. When assembling the evaporation manifold, it is important that the holes melted into the cultureware are of uniform size/diameter in order to allow even distribution of the gas flow and to produce uniform solvent evaporation in wells throughout the culture plate. Wetting the surface of the flask ensures that the application of the pipette tip/superglue complex will adhere. It is also important to verify that the pipet tips have not become plugged with superglue after fixing them to the flask. If a pipet tip dislodges, a new one can be prepared easily and reglued to the manifold. As long as the apparatus is handled in a limited manner, there is little need for tip reassembly.

Comparison to other evaporation units. A number of commercially available evaporation systems are capable of achieving similar results, but they are more costly than our small number of samples warranted. Commercially available evaporators are priced from $495.00 to $2285.00. The estimated cost for this apparatus including labor is around $130.00. Due to its low cost and ease of construction, this manifold may be of interest to small laboratories with minimal operation budgets.

References

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