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SPECIMEN HOLDERS FOR SIMULTANEOUS CRITICAL POINT DRYING OF MULTIPLE BIOLOGICAL SPECIMENS

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ABSTRACT Specimen holders were developed for simultaneous critical point drying of multiple specimens of monolayer cell cultures grown on Leighton tube glass cover slips or for specimens of cells collected on silver or cellulose membrane filters. The use of these multiple specimen holders makes it possible to process several specimens in parallel and thus significantly reduce the time required for handling large numbers of samples and also eliminate the possible variations that occur in processing individual samples one at a time in series.

This laboratory is engaged in testing of polymeric materials for biodegradation and biocompatibility and in the development of in vitro methods for prescreening of these materials for any bio-interaction. In an effort to correlate the possible effects of sublethal doses of toxic polymeric degradation products on cell morphology and ultrastructure, we have used the critical point drying method of Anderson (1951) or a modification of it (De Bault 1973, Lewis and Nemanic 1973). This is the mildest method known for biological specimen drying and is considered to induce only minimal shrinkage and surface alterations (Boyd 1971).

In our in vitro screening system, test substances are placed into monolayer cell cultures grown on Leighton tube glass cover slips. At the end of the test period, most of the cells are still attached to the cover slips; however, depending upon the extent of cytotoxicity of the test material, some cells are detached and float freely in the medium. These free floating cells are collected on Flotronics (Selas Corporation of America, Spring House, PA) or Millipore (Millipore Corporation, Bedford, MA) filters. Because of the lack of availability of multiple specimen holders for critical point drying of cells grown on Leighton tube cover slips, and since the holders for membrane filter preparations from the different manufacturers were not the proper size to fit the Parr drying bomb, two different types of holders were constructed in our laboratory. The two holders discussed in this article were constructed to hold several of either the glass cover slips or the filters during the critical point drying process.

The holder in Figure 1 can be fitted into the Parr 4770 critical point drying apparatus. It holds 22 glass cover slips, 9 mm x 50 mm and was made from two pieces of ⅜ x 2 x 2 inch flat stock machined to obtain the desired dimensions. The two halves were welded together and then welded to a 2⅛-inch diameter circular bottom. A pin was placed through the bottom of the holder to keep the cover slips from falling through. The two solid side pieces were machined from 2⅛-inch diameter stock and were incorporated into the design to reduce dead space and thus the amount of liquid used. There is a ⅛-inch diameter hole in one side piece to accommodate the exhaust.
Our routine for critical point drying with these holders is as follows: The biological material on the glass slides or Flotronics filters is placed in the special holder, fixed in 2% glutaraldehyde for 2 hr, washed with 0.15 M phosphate buffer, fixed in 2% osmium tetroxide at 0°C for 30 min, washed twice with 0.15 M phosphate buffer, and dehydrated with ethanol (50%, 75%, 95%, 100%, 100%) in 10-min steps. The ethanol is then replaced in 10-min steps with increasing concentrations of isooamyl acetate (50%, 75%, 95%, 100%, 100%).
The samples in the holder are then transferred to a Parr critical point drying apparatus and critical point dried with liquid CO₂ as the transitional fluid, according to the method described by Anderson. Approximately 9 × 9 mm squares of either the Flotronics filters or the glass cover slips are mounted on aluminum stubs with All Purpose Cement No. 527 (Bond Adhesives Co., Jersey City, N.J.) and conductive paint, and evaporation coated with carbon and gold in a VE 10 vacuum evaporator (Varian Vacuum Division, Palo Alto, CA) at 2 × 10⁻⁵ Torr (Echlin 1974). The specimens are examined in a Model 1000 scanning electron microscope (Advanced Metals Research Corp., Burlington, MA) with an accelerating voltage of 20 kV and a resolution of 100-200 Å. Samples collected on Millipore filters are soluble in this intermediate fluid, and are therefore critical point dried according to De Bault's modification of Anderson's method.

We have obtained excellent results using these two holders for the critical point drying process. The use of these holders has decreased the time necessary for processing multiple samples.

Another advantage of processing samples in these holders is that any differences between individual sample preparations due to minor variations in procedure are eliminated.
**REPORT DOCUMENTATION PAGE**

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<th>1. REPORT NUMBER</th>
<th>2. GOVT ACCESSION NO.</th>
<th>3. RECIPIENT'S CATALOG NUMBER</th>
<th>4. TITLE ( electric)</th>
<th>5. STATE OF REPORT &amp; PERIOD COVERED</th>
<th>6. PERFORMING ORG. REPORT NUMBER</th>
<th>7. CONTRACT OR GRANT NUMBER(s)</th>
<th>8. CONTROLLING OFFICE NAME AND ADDRESS</th>
<th>9. PREPARATION DATE</th>
<th>10. NUMBER OF PAGES</th>
<th>11. REPORT DATE</th>
<th>12. NUMBER OF PAGES</th>
<th>13. MONITORING AGENCY NAME &amp; ADDRESS</th>
<th>14. SECURITY CLASS. (of this report)</th>
<th>15. SECURITY CLASS. (of this report)</th>
<th>16. DISTRIBUTION STATEMENT (of this Report)</th>
</tr>
</thead>
<tbody>
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<td>SPECIMEN HOLDERS FOR SIMULTANEOUS CRITICAL POINT DRYING OF MULTIPLE BIOLOGICAL SPECIMENS</td>
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<td></td>
<td>In vitro Testing</td>
<td>US Army Medical Bioengineering Research &amp; Development Laboratory, Bldg. 568, Ft. Detrick, Frederick, MD 21701</td>
<td>15. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)</td>
<td>Published in STAIN TECHNOLOGY 51: No. 1, pp 51-53 (1976)</td>
<td>Scanning electron microscopy Critical point drying Tissue culture</td>
<td>Specimen holders were developed for simultaneous critical point drying of multiple specimens of monolayer cell cultures grown on Leighton tube glass cover slips or for specimens of cells collected on silver or cellulose membrane filters. The use of these multiple specimen holders makes it possible to process several specimens in parallel and thus significantly reduce the time required for handling large numbers of samples and also eliminate the possible variations that occur in processing individual samples one at a time in series.</td>
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