VANASYL LLC.
The VANASYL Sampling Valve

The Sampling Valve for the Bio/Pharmaceutical Industry

THE SAMPLING SOLUTION
The operation of the Vanasyl Sampling Valve is described below:

**Sterilisation Stage (Forward Position):**
With the handle fully forward (toward the vessel) the front seal (A), which seals the vessel, is flush with the front of the sampling valve eliminating dead space.
Steam enters the valve via the inlet, flows between the two seals and exits via the condensate/sample outlet.
The Vanasyl Sampling Valve should always be left in this position when not in use.

**Shut Off Stage (Mid-Position):**
Before taking a sample the valve's handle is pulled back (toward the operator). It will automatically lock in the mid-position.
In the mid-position, the front seal closes the outlet line, stopping the steam flow while the vessel remains shut.
At this stage, while allowing the valve to cool down, the outlet line could be switched from condensate to sample.
Condensate will accumulate between the seals.

**Sampling Stage (Rear Position):**
After lifting the valve handle, which will unlock it from the mid-position, it should be pulled back to the rearmost position.
The front seal moves from the outlet line allowing the sample from process to flow through the outlet line into a suitable container (bottle, etc.).
Having taken the sample, the handle should be returned to the mid-position, and the condensate line reattached if required. The valve handle should then be brought to the steam sterilising position.
The accumulated condensate flushes the valve outlet line and the sterilisation stage begins (1 above).
A. STEM SEAL PERFORMANCE

The use of two seals verified that the sealing performance was satisfactory when pressure was applied to either side of the seal.

1. Nitrogen Tests (Oxygen-free nitrogen at room temperature)

Various tests were performed at pressures between 17 and 36 bar (250 and 520 psi), lasting up to 2300 cycles, with no detectable leaks. In these tests two cycling schemes were adopted:

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Stroke</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 x 15mm</td>
<td>300 cycles/hour</td>
</tr>
<tr>
<td>2</td>
<td>2 x 22mm</td>
<td>100 cycles/hour</td>
</tr>
</tbody>
</table>

Each test was concluded by raising the pressure to a maximum of 76 bar (1100 psi), confirming the integrity of the seal.

Tests with differing grades of PTFE as the seal material showed that a light carbon/graphite-filled PTFE was better suited to higher cycling rates.

'Snoop®' (a special foaming liquid designed for the purpose) was used for leak detection. A comparison with helium gas leak detection indicates that leaks at the rate of $10^{-6}$ Std cc/sec could be detected.

2. Steam Tests

Self-contained steam-generation equipment was used to circulate steam through the test chamber at pressures up to 15 bar (220 psi) and temperatures up to $200^\circ C (392^\circ F)$.

Leak-free performance was confirmed to substantially more than 2000 cycles.

3. Steam Endurance Test

A steam endurance test was conducted, using circulating steam at pressures up to 15 bar (220 psi) and temperatures up to $200^\circ C (392^\circ F)$.

After completing 104,800 cycles with no leak, the steam generator broke down, and the seals were taken out for inspection. The following observations were made:

a. The seals showed virtually no wear.

b. The internal surfaces of the test chamber were covered in a brown deposit in all areas except those over which the seals moved, where there was clear evidence of PTFE having become embedded. Investigation revealed that the deposit was copper which, under the conditions of the test, had leached from the brass test components and had coated the exposed surfaces.

(It should be noted that the presence of brass was overlooked.)

This unplanned episode yields valuable information about the behaviour of the seals since the areas which they traversed were free of such deposits. This indicated that the wiping action of the seal did indeed clean the mating surface of the bore.

Furthermore, it indicated that foreign material does not adhere to PTFE-coated surface, eliminating damage to the seal, a main cause to a reduction in seal performance.

4. Helium Tests

See Helium Leak Test Data sheet HTL-150.
B. VALVE PERFORMANCE

An electrically actuated valve was assembled without its obturator (plug).

1. Steam

The prototype valve was connected to the steam test-rig and was operated continuously in a 2 minute open/close cycle, sealing steam at 8.5 bar (120 psi) and 175°C (350°F). Throughout the test, lasting 5300 cycles, the seal was monitored for leaks; none was detected.

2. Microbiological Material

The valve was then installed in a secure microbiological test rig within the Centre for Applied Microbiology and Research at the Public Health Laboratory Service facility at Porton-Down in England.

Spores of 'bacillus subtilis var. Globigii' at a concentration of $8 \times 10^9$ colony-forming units per millilitre were circulated through the valve. The valve's purge ports were connected to a 'Slit Sample', and the seal was monitored for leaks. In each daily test period 60 samples of 20-litre air were taken. The Petri Dish was then allowed to incubate for 24 hours at 37°C.

During the 5-day working week the valve was cycled at a rate of 60 cycles per hour. During the weekend the valve was left in its open position to ascertain if any growth occurred across the seal during long periods of no seal movement.

After over 7,200 cycles the test was completed with no indication that any spores had crossed the seal.

C. SEAL WEAR

The seal that was used in the 12,500 cycle microbiological test described above was weighed before and after the test, and the results were:

Weight at start (g): 17.5495
Weight at end (g): 17.5470
Difference (g): 0.0025
Difference as %: 0.014%

It is difficult to judge when the wear occurred during the long duration of the test but it is probable that a substantial proportion took place during the 'bedding-in' process, that is, the initial few valve cycles when the seal is compressed against a newly-honed valve body.

_________________________________________________________________________________

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Clifton Enterprises is the trading name of Vanasyl Ltd.
TEST SYSTEM
The test system comprised a 15 litre commercially available Spin-filter bioreactor used for long term mammalian cell culture.

AIMS
a) To test the suitability of use and operation of the valve for cell culture sampling.
b) To test whether sterility is compromised by use of the new Vanasyl Valve.

STERILITY TESTS
The following tests were carried out:
1) 5ml samples of sterilised nutrient broth were taken repeatedly for 22 cycles. The valve was sterilised between each sampling.
2) Large sample volumes were taken to test for the effect of long sampling times and larger volumes. These included 50ml samples, 100ml samples and 400ml samples.

The sterility of the samples was tested by incubating the samples in nutrient broth (N/B), thioglycollate broth (T/B), nutrient agar (N/A) and sabouraud dextran agar (S/A) in a 37°C hot room.

STERILISATION OF THE BIOREACTOR
The bioreactor was thoroughly cleaned with 7X Phosphate-free detergent used in animal cell culture and MilliQ water. The reactor was sterilised containing 11 litres MilliQ water at 121.5°C for 45 minutes. The reactor was cooled down to 37°C after sterilisation.

The harvest port in the reactor was sterilised for 15 minutes using steam generated from the steam generator in the reactor. The water remaining in the reactor was drained via the sterilised harvest port and 11 litres of sterile nutrient broth was added aseptically.

An indication of the sterility of nutrient broth in the reactor was gained for the pH and DO$_2$ profiles recorded during the test period.

STERILISATION OF THE VANASYL VALVE
A silicone tube was attached to the sample outlet of the Vanasyl valve in order to retain back pressure during sterilisation. The Vanasyl valve was sterilised with steam, generated from the steam generator supplied with the reactor, for 15 minutes.

RESULTS
a. Sterility test after sampling
The sample sterility tests were incubated in 37°C hot room for two weeks. The sterility was recorded every week. After two weeks incubation all the sample tests were clear. (See attached sterility tests record.)

b. Sterility of the reactor
The sterility of the reactor was maintained after sterilisation and during the sampling period. (See the pH and DO$_2$ profiles in the reactor test results table.)
CONCLUSION

Independent operators noted that the Vanasyl Valve is very easy to operate compared to the previous design in the reactor and passed all the sterility test procedures that were carried out. The sampling and sterilisation sequences carried out are representative of the techniques used for sampling in animal cell culture reactors.

The Vanasyl Valve is highly recommended for use in animal cell culture reactors, because of its ease of use and high performance.

SAMPLING RESULTS

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mls)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>50</td>
<td>5</td>
</tr>
</tbody>
</table>

Key:  p = pass,  f = fall

Larger sample volumes were tested using an identical protocol. No failures were recorded for these larger samples.

pH and DO₂ Profiles in the Reactor During the Test Period:

<table>
<thead>
<tr>
<th>Culture</th>
<th>Time (day)</th>
<th>pH</th>
<th>DO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.05</td>
<td>83.6%</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.10</td>
<td>82.1%</td>
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</tr>
<tr>
<td>2</td>
<td>7.07</td>
<td>85.3%</td>
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<tr>
<td>3</td>
<td>7.02</td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>7.09</td>
<td>84.8%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.07</td>
<td>85.7%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7.10</td>
<td>89.0%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7.03</td>
<td>85.1%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.06</td>
<td>84.6%</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>7.01</td>
<td>88.3%</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7.04</td>
<td>84.2%</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>7.07</td>
<td>83.3%</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>7.08</td>
<td>85.5%</td>
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<tr>
<td>13</td>
<td>7.06</td>
<td>83.7%</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>7.04</td>
<td>85.3%</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>7.01</td>
<td>88.5%</td>
<td></td>
</tr>
</tbody>
</table>

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The Vanasyl Sampling Valve CIP & SIP Procedures.

A. In normal use, the Vanasyl Sampling Valve requires no special CIP cycle after sample collection, and between batches, as long as the valve is brought to the steam sterilisation position immediately after the collection of sample.

This is due to the fact that during shut off and sampling positions, the condensate, which is collected behind the front seal, flushes the valve’s condensate/outlet port prior to the introduction of steam once the valve is brought to the ‘Steam Sterilisation Position’.

B. It is advisable to replace the Ingold O-ring between batches. This should be done after the vessel’s CIP cycle, during which, the valve should be left in the Steam Sterilisation Position.

The Ingold O-ring grove must be free of the cleaning solutions whether or not a new O-ring is used.

THE VALVE SHOULD NEVER BE:

1. Disassembled. Extracting the stem-seals-assembly could cause damage, which would require the re-honing of the valve body and the replacement of the stem-seals-assembly.

2. Soaked in cleaning fluids. This will cause irreparable damage to the valve.

For clarification and further information, please contact:

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The Vanasyl Sampling Valve Maintenance Schedule

1. The valve itself requires no maintenance. Extracting the stem seal assembly will irreversibly damage it and could damage the valve body.

2. For CIP & SIP procedures please refer to ‘Clifton Enterprises Technical Note No.: 5333A’.

3. The only parts that should be regularly replaced are the O-Rings. Clifton Enterprises cannot dictate the schedule for the O-rings replacements however, we would recommend that the:
   a. Ingold O-ring\(^1\) should be replaced after every batch (See TN 5333A section B).
   b. Inlet\(^2\) and Outlet\(^3\) O-rings should be replaced at least twice a year.

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\(^1\) Clifton Enterprises part No.: 00-215 [FDA Approved EPDM (20.29 x 2.62mm)]
\(^2\) Clifton Enterprises part No.: 00-217 [FDA Approved EPDM (3.17 x 1.78mm)]
\(^3\) Clifton Enterprises part No.: 00-216 [FDA Approved EPDM (8 x 2.5mm)]
The Transfer Layer Principle, Non-Polishing
and the Vanasyl Sampling Valve

A. The unique properties (e.g. low coefficient of friction, chemically inert, etc.) of Polytetrafluoroethylene (PTFE, also known in its trade name Teflon), are due to the Fluorine Element (F) within the PTFE molecule. These properties make the PTFE, in some circumstances, a choice material for the use in bearing and seals material. However, when PTFE is used as a bearing or as a dynamic seal against polished stainless steel, the PTFE releases some of its F atoms, which immediately attacks the stainless steel causing extreme and irreversible damage. This is why we do not polish.

B. The only way that the unique properties of PTFE can be utilised, is if some of the PTFE is transferred to the stainless steel surface. The bore of the Vanasyl Valve body is honed in a specific way to enable the PTFE from the seals to be transferred to the bore of the Vanasyl Valve body. The PTFE layer that is embedded in the stainless steel surface is known as the ‘transfer layer’, which protects the stainless steel as well as reduces the friction between the mating components.

C. After the seals subassembly is inserted into the Vanasyl Valve bore, the valve is then operated for over 100 times between the steam and sample positions, to create the ‘transfer layer’. The valve is then pressure-tested ensuring the seals integrity.

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Changing Handle-Orientation of the Vanasyl Sampling Valve

1) When the valve is out of the vessel or for changing the handle-orientation in situ, the handle should be in the STEAM Position (the handle towards the valve body) and NEVER IN THE OFF OR SAMPLING POSITION.

2) Using a 2.5mm Allen Key, remove the 3 socket-screws (the use of any other tool WILL damage the socket-screws).

3) Rotate the handle only in a CLOCKWISE DIRECTION to the chosen orientation.

4) If during the rotation of handle the holes on the fulcrum are to be found to the left of the valve’s body threaded holes, the handle should be rotated CLOCKWISE DIRECTION (nearly 360° to the chosen position) to properly align the holes. THE HANDLE SHOULD NEVER BE ROTATE (EVEN SLIGHTLY) IN AN ANTICLOCKWISE DIRECTION!

5) Insert the 3 socket-screws using a 2.5mm Allen Key to ensure that they are properly threaded and secured.

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Benefits of Sampling Using the Vanasyl Sampling System

- Very low maintenance required (O-ring replacement).
- No risk of cross contamination.
- Faster sampling. A sample can be taken, anywhere from 10 to 30 seconds after filling the sampling bag via a sterile syringe ensuring sample integrity and reliability.
- Self-cleaning
- No needles- eliminating needle injuries and disposal issues.
- Sampling bags are made from EVA.
- Bag can be stored, then frozen down to -120°C, and then brought back to room temperature and samples can continue being taken.
- UV protected.
- No need to empty the bag.
- No need for crimping- minimizing human error.
- No need for a safety cabinet.
- Bags pre-sterilized (gamma-irradiated).
- No particulates.
- No leakage.
- Non-fragile.
## Low Temperature Storage of the Vanasyl Sample Bags

<table>
<thead>
<tr>
<th>Date</th>
<th>Bag 1 wt gm</th>
<th>Bag 2 wt gm</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>18/2/14</td>
<td>162.0, 162.0, 162.0</td>
<td>157.3, 157.3, 157.3</td>
<td>Samples weighed then placed in -80 freezer</td>
</tr>
<tr>
<td>21/2/14</td>
<td>162.0, 162.0, 162.0</td>
<td>157.3, 157.3, 157.3</td>
<td>Samples defrosted equilibrated to room temp then weighed returned to freezer</td>
</tr>
<tr>
<td>3/3/14</td>
<td>162.0, 162.0, 162.0</td>
<td>157.3, 157.3, 157.3</td>
<td>Samples defrosted equilibrated to room temp then weighed returned to freezer</td>
</tr>
<tr>
<td>7/3/14</td>
<td>162.0, 162.1, 162.0</td>
<td>157.3, 157.3, 157.3</td>
<td>Samples defrosted equilibrated to room temp then weighed samples placed in -93 freezer</td>
</tr>
<tr>
<td>13/3/14</td>
<td>161.9, 161.9, 161.9</td>
<td>157.3, 157.3, 157.3</td>
<td>Samples defrosted equilibrated to room temp then weighed</td>
</tr>
<tr>
<td>18/3/14</td>
<td>159.7, 159.7, 159.7</td>
<td>156.0, 155.9, 155.9</td>
<td>Samples defrosted equilibrated to room temp volume removed then weighed replaced in -93 freezer</td>
</tr>
<tr>
<td>21/3/14</td>
<td>159.6, 159.7, 159.6</td>
<td>155.9, 155.9, 155.9</td>
<td>Samples defrosted equilibrated to room temp weighed replaced in -93 freezer</td>
</tr>
<tr>
<td>28/3/14</td>
<td>158.3, 158.4, 158.4</td>
<td>154.8, 154.8, 154.8</td>
<td>Samples defrosted equilibrated to room temp volume removed then weighed replaced in -93 freezer</td>
</tr>
<tr>
<td>11/4/14</td>
<td>158.3, 158.3, 158.3</td>
<td>154.8, 154.8, 154.8</td>
<td>Samples defrosted equilibrated to room temp placed in -150 approx. vapour above liquid N2</td>
</tr>
<tr>
<td>17/4/14</td>
<td>158.3, 158.3, 158.3</td>
<td>154.7, 154.8, 154.8</td>
<td>Samples defrosted equilibrated to room temp weighed returned to -150 approx tank.</td>
</tr>
<tr>
<td>22/4/14</td>
<td>158.0, 158.0, 158.0</td>
<td>154.5, 154.6, 154.6</td>
<td>Samples defrosted equilibrated to room temp weighed returned to -150 approx tank.</td>
</tr>
<tr>
<td>28/4/14</td>
<td>157.8, 157.8, 157.8</td>
<td>154.3, 154.3, 154.3</td>
<td>Samples defrosted equilibrated to room temp weighed stored at room temp.</td>
</tr>
<tr>
<td>15/5/14</td>
<td>157.6, 156.7, 157.6</td>
<td>153.9, 153.9, 153.9</td>
<td>Weighed at room temp.</td>
</tr>
<tr>
<td>28/5/14</td>
<td>156.7, 156.7, 156.7</td>
<td>153.1, 156.1, 153.1</td>
<td>Weighed at room temp.</td>
</tr>
</tbody>
</table>

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Professional Services  
Medical Physics  
Department of Cardiovascular Science  
University of Sheffield
**Technical Data for Multilayer Ethylene-Vinyl Acetate (EVA) with Barrier**

*(Film Gauge 400 μm @ 23 °C)*

**EVA Without a Barrier**

<table>
<thead>
<tr>
<th>Physical Properties</th>
<th>Dimension</th>
<th>Value</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Vapour Transmission Rate</td>
<td>g/(m².day)</td>
<td>1.64</td>
<td>ASTM F-1249</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen Permeability</td>
<td>cm²/(m².day.bar)</td>
<td>2.2</td>
<td>ASTM D-3985</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon Dioxide Permeability</td>
<td>cm²/(m².day.bar)</td>
<td>6</td>
<td>Mocon Permatran C-IV</td>
</tr>
<tr>
<td></td>
<td>7000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV Transmission</td>
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<td></td>
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</tr>
</tbody>
</table>
THE VANASYL SAMPLING SYSTEM:

The Vanasyl Sampling System can be bought either through our UK company (Clifton Enterprises/Vanasyl LTD) or through our US company (Vanasyl LLC)

OUR PRODUCTS:

Our products consist of stainless steel sampling valves, various adaptors, if requested, and polymeric bags. See items below:

- SV-123 The Vanasyl Sampling Valve
- SV-260-10-A Bag Connection
- SB Series Bags (250ml, 500ml, and 1L)
- 00-216 O-rings
- SV-3XX Various Spacer Adaptors (need depending only on length of Ingold connection).
- SV-402/403,451/45X Various Inlet/Outlet Adaptors (need only with tri-clamp/tube connection)

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