Aseptic Sampling Best Practices:
Sample Collection for pH and Gas Analysis

Introduction

Accurate monitoring of dissolved oxygen, carbon dioxide and pH is essential to a successful drug production process. The most precise results begin with how the sample is collected.

Syringe: Ideal for pH and Gas Analysis

Syringes are an ideal sample collection vessel for pH, DCO₂ and DO₂ analysis. Sartorius consulted with industry leading bioanalyzer manufacturer Nova Biomedical® to detail why.

Time From Sample to Analysis

The pH of a sample will shift as cells within consume O₂ and produce CO₂. The longer the time between sample collection and analysis the less accurate the analysis test will be. Samples collected into a syringe can be taken directly to the analyzer for testing, giving the most precise result.

Gas Exchange

De-gassing or off-gassing occurs where the fluid is in contact with a surface or the environment. Cylinders, like a syringe, maintain a more stable surfacearea-to-volume ratio. Gas exchange is limited and stabilized.
The Chemistry of pH

1. When carbon dioxide dissolves into water it exists in equilibrium to form carbonic acid:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

2. Carbonic acid disassociates to generate a free hydrogen ion which can change a solution’s pH:

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

Environment

Saturation of $O_2$ and $CO_2$ in cell culture can be different from saturation levels in air. Gases in air bind to the plastic surface of the sample collection container prior to use. The bound gases transfer from high to low concentration artificially changing dissolved gas in the sample.

Syringes are not immune to the effect but the more stable surface area-to-volume ratio reduces its impact.

Stable Surface Area to Volume Ratio

There is more opportunity for gas exchange where the solution is in contact with a surface. Consequently, there is less variability of gas exchange with stable surface area-to-volume ratios.

<table>
<thead>
<tr>
<th>Sample Volume Collected</th>
<th>10 mL Syringe</th>
<th>50 mL Bag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surface Area [cm²]</td>
<td>Surface Area to Volume</td>
</tr>
<tr>
<td>2mL</td>
<td>8.82</td>
<td>4.41</td>
</tr>
<tr>
<td>5 mL</td>
<td>17.10</td>
<td>3.42</td>
</tr>
<tr>
<td>10 mL</td>
<td>30.89</td>
<td>3.09</td>
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</tbody>
</table>

Materials of Construction

Syringes are constructed of plastic. All plastics will allow some degree of gas transmission into or out of solution. The slower the rate of gas transmission, the more accurate the gas or pH analysis will be.

Syringes are constructed of thick-walled and rigid plastic. Compared to thin-walled flexible plastics gas transmission rates are slowed in a syringe.
Step-by-Step Instructions for Use

1. Sanitize valve surface
2. Insert fitting straight into valve
3. Turn clockwise to engage

BENCHMARK™ Sampling Line
- Precise pH, DO₂ & DCO₂ sample analysis
- High frequency sampling from a single sampling line
- Economical solution using conventional equipment and components

Description
BENCHMARK™ features a luer-activated access site which accepts any standard male luer fitting – common to most syringes.

The stem of the male luer opens the silicone valve as it is connected to the access site allowing for unimpeded fluid flow. The valve closes when the male luer is disengaged.

Secure Expansion of Sampling Capacity
The access valve has been validated to maintain a microbial barrier after 140 actuations making it suitable for high-frequency sampling requirements.

Applications
- BENCHMARK™ is ideal for most process-monitoring sampling needs
- Configure BENCHMARK™ on the Takeone® aseptic sampling system
- Connect BENCHMARK™ to benchtop bioreactors