# Evaluation of Rapid Automated pH Measurement Technology for Commercial Sterility Testing

**Micah Greenzweig, Julie Weller, Nate Banner and Anne Roelfing**
Hygiena®, 2 Boulden Circle, New Castle, DE 19720

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## INTRODUCTION:

Measurement of pH is widely used as an indicator of potential contamination during the production of commercially sterile shelf-stable liquid food and beverage products. As microorganisms metabolize nutrients within the product, they often produce organic acids and other byproducts that will alter the product's normal pH.

The extent of the pH change can vary depending on factors such as product composition (e.g., sugar content, buffering capacity and preservatives) as well as the type of microorganisms present. In cases of minor contamination, the pH may decrease 0.3-0.5 units, whereas active spoilage can result in a more pronounced reduction of 1.0 – 3.0 units.

Regardless of the magnitude of the change, microbial growth disrupts the product's normal stable pH and can serve as an early indication of spoilage.

## PURPOSE:

The objective of this study was to evaluate the suitability of a rapid automated pH system for use in commercially sterile liquid food and beverage products with the following goals:

1. Assess the system's ability to generate accurate and reproducible pH measurements compared to conventional manual methods.
2. Evaluate potential cross-contamination risks.
3. Evaluate the sensitivity of microbial-induced pH changes across diverse product matrices.

## REGISTERED TRADEMARKS:

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## PHASE 1:

**Method**
A range of product types, including chicken broth, plant-based milks, protein shakes, and viscous matrices such as cheese sauce and chocolate pudding, were pre-incubated at 32 to 33 °C for 48 hours. Following incubation, the pH was measured with both the rapid automated pH meter and a standard benchtop pH meter. For each matrix, an additional portion was adjusted with hydrochloric acid (HCl) to approximately 2.0 units below the typical pH range to test performance across a broader range.

**Results (Table 1)**
- Automated and manual pH readings aligned across direct and acid-adjusted conditions.
- Variation across matrices were minimal, with slight differences in absolute pH values.

**Table 1: Comparison of pH Measurements with Product Matrix Panel**

| Product Matrix | Direct | | | | | | | Acid Adjusted | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rapid | | | Manual | | | % Difference | Rapid | | | Manual | | | % Difference |
| | Avg | Std Dev | CV% | Avg | Std Dev | CV% | | Avg | Std Dev | CV% | Avg | Std Dev | CV% | |
| Chicken Broth | 5.54 | 0.238 | 4% | 5.72 | 0.053 | 1% | 3.09% | 2.84 | 0.126 | 4% | 2.87 | 0.202 | 7% | 1.25% |
| Vanilla Soy Milk | 8.56 | 0.03 | 0.4% | 8.26 | 0.089 | 1% | 3.52% | 6.81 | 0.37 | 5% | 6.45 | 0.412 | 6% | 5.29% |
| Oat Beverage | 7.76 | 0.05 | 0.7% | 7.32 | 0.09 | 1% | 5.60% | 4.58 | 0.521 | 11% | 4.41 | 0.505 | 11% | 3.78% |
| Chocolate Collagen Shake | 7.41 | 0.107 | 1.4% | 6.57 | 0.049 | 1% | 11% | 5.84 | 0.163 | 3% | 4.78 | 0.123 | 3% | 18% |
| Chocolate Pudding (1:1 Dilution) | 7.48 | 0.047 | 0.6% | 6.32 | 0.095 | 2% | 15.58% | 5.59 | 0.207 | 4% | 4.52 | 0.111 | 2% | 19% |
| Cheddar Cheese Sauce (1:1 Dilution) | 6.56 | 0.064 | 1% | 5.62 | 0.204 | 4% | 14% | 4.94 | 0.667 | 13% | 3.68 | 0.239 | 6% | 25% |

## PHASE 2:

**Method**
Chicken broth was divided into two aliquots: one untreated (normal pH) and one acidified with HCl to reduce the pH by approximately 3.0 units. Samples were then dispensed onto a 96-well microtiter plate in an alternating checkerboard pattern to assess the potential for cross-contamination during automated measurement.

**Results**
- No evidence of cross-contamination between adjacent wells.
- Replicate variability remained low (< 0.10 pH units) within an acceptable range.

**Untreated**
Mean = 5.19
Std deviation = 0.084

**Acidified**
Mean = 2.75
Std deviation = 0.060

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | 5.23 | 2.68 | 5.11 | 2.75 | 5.14 | 2.75 |
| B | 2.71 | 5.09 | 2.72 | 5.11 | 2.70 | 5.11 |
| C | 5.15 | 2.74 | 5.29 | 2.75 | 5.26 | 2.76 |
| D | 2.72 | 5.29 | 2.76 | 5.31 | 2.77 | 5.14 |
| E | 5.09 | 2.98 | 5.26 | 2.80 | 5.27 | 2.75 |
| F | 2.68 | 5.29 | 2.78 | 5.16 | 2.78 | 5.13 |
| G | 5.11 | 2.79 | 5.11 | 2.70 | 5.28 | 2.73 |
| H | 2.72 | 5.34 | 2.79 | 5.13 | 2.79 | 5.14 |

## PHASE 3:

**Method**
Beef broth and oat milk were inoculated with a variety of spoilage organisms and incubated at 32 to 33 °C for 3-5 days. On day 3 and day 5, aliquots were aseptically removed and the pH was measured with both the rapid automated pH meter and a standard benchtop pH meter. Conventional agar plating was performed in parallel to confirm microbial growth.

**Results**
- Detectable pH drops associated with microbial spoilage were observed compared to uninoculated controls.
- For some combinations of matrix and organism, pH changes were not clearly distinguishable.
- All inoculated samples were confirmed positive by culture.

**Beef Broth**

| Organism | Incubation Time (Days) | Average pH | | Difference Compared to Negative | |
|---|---|---|---|---|---|
| | | Rapid | Manual | Rapid | Manual |
| Negative Control | 3 | 5.88 | 6.12 | - | - |
| | 5 | 6.08 | 6.04 | - | - |
| *Enterococcus casseliflavus* 2.3 CFU | 3 | 5.33 | 5.60 | 0.56 | 0.52 |
| | 5 | 5.53 | 5.67 | 0.55 | 0.37 |
| *Staphylococcus aureus* 6.8 CFU | 3 | 5.55 | 5.51 | 0.33 | 0.60 |
| | 5 | 5.92 | 5.98 | 0.16 | 0.07 |
| *Salmonella* Enteritidis 8.3 CFU | 3 | 6.15 | 6.44 | 0.27 | 0.32 |
| | 5 | 6.74 | 6.42 | 0.66 | 0.38 |

**Oat Milk**

| Organism | Incubation Time (Days) | Average pH | | Difference Compared to Negative | |
|---|---|---|---|---|---|
| | | Rapid | Manual | Rapid | Manual |
| Negative Control | 3 | 7.66 | 6.87 | - | - |
| | 5 | 7.10 | 7.33 | - | - |
| *Clostridium sporogenes* 33.8 CFU | 3 | 6.67 | 6.07 | 1.00 | 0.80 |
| | 5 | 5.73 | 5.91 | 1.37 | 1.42 |
| *Saccharomyces cerevisiae* 10.4 CFU | 3 | 7.45 | 6.85 | 0.21 | 0.02 |
| | 5 | 6.51 | 6.04 | 0.58 | 1.29 |
| *Salmonella* Typhimurium 9.6 CFU | 3 | 5.67 | 5.27 | 2.00 | 1.60 |
| | 5 | 4.80 | 4.94 | 2.29 | 2.39 |

## SIGNIFICANCE:

**The rapid automated pH meter is accurate, reliable and suitable for diverse matrices and conditions.**

**Demonstrates robust performance with no cross-contamination and low variability.**

**Effectively detects microbial spoilage, comparable to manual pH readings and plate confirmation.**

No. 10 Envelope