24-Month Post-Gamma Qualification of Single-Use pH Sensors in Single-Use Bioreactor, Mixer, and Fermentor Equipment

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Bioprocessing

ABSTRACT

Purpose: Determine if Hamilton OneFerm™ sensors have sufficient gamma stability, low drift, long shelf life, and ease of integration in single-use bioreactors (S.U.B.), fermentors (S.U.F.), and mixers (S.U.M.).

Methods: Six Hamilton OneFerm™ VP 70 pH probes were installed in a standard 50L cell culture Bio-Process Container (BPC) and gamma sterilized. The BPC was then aged for 24 months, adhering to proper storage guidelines. A 21 day cell culture was then performed followed by the sensors being stored in the culture broth for 39 more days. Finally, after a total of 60 days in culture media, the probes were removed and tested for response time.

Results: All sensors tested performed within the manufacturer's specifications.

INTRODUCTION

The implementation of new process analytic tools is consistent with Thermo Fisher Scientific's continuous improvement process and open architecture support. Hamilton Company's OneFerm™ pH VP 70 sensor (HC part # 238999-4955 and TFS part # SV21484.01) is a single-use glass electrode that provides an additional option for customers wishing to implement single-use pH sensing capabilities in the S.U.B., S.U.F, and S.U.M.

The OneFerm™ probes were tested against manufacturer specifications to ensure a robust option even after 24 months of dry storage post-irradiation.

To incorporate the Hamilton OneFerm™ within the Thermo Fisher Scientific single-use BPCs, a custom port adapter (part SV21527.01) was selected as the most robust method for integration into the BPC. After integration with the BPC, the sensors were gamma irradiated, and stored for 24 months, before being evaluated in a rigorous cell culture based test.

Figure 1. Image of OneFerm™ sensor installed in a S.U.B. BPC

Three versions of the single-use OneFerm™ sensors are available for order in a BPC: SV21484.01 (PT-1000 type), SV21484.02 (PT-100 type), and SV21484.03 (NTC type for ARC™).

MATERIALS AND METHODS

Cell Culture

Cell culture was performed according to a typical in-house fed-batch process[1]. The media components were Gibco™ Dynamis™ AGT™, supplemented with 45% glucose and EfficientFeed™ C+ 2x. Agitation was a constant 20 W/m^3 (186 RPM). The pH was controlled using sparged CO_2, while targeting 30-80 mmHg of CO_2. The pH setpoint was variable: day 0-3 ramped from 7.2 to 7.0, days 3-21 a constant 7.0 but allowing to drop to 6.8, no bases or acids were used. DO was controlled with a constant headspace air flow of 2 slpm, with variable O_2 and N_2 through the Drilled-Hole Sparger (DHS). An 8 day feed of EfficientFeed™ C+ started on day 3 with glucose supplemented as necessary. CHO-S 5B9 were the cells cultured in this experiment. Antifoam C was used as needed.

Test Methods

BPCs were created using Thermo Fisher Scientific production standards. Each BPC was built to include six OneFerm™ sensors, two each from three different manufacturer lots. The BPCs were subsequently sterilized using gamma irradiation (single dose, 25-40 kGy) and subjected to a 24 month aging period. A new Hamilton EasyFerm™ pH sensor (sterile autoclaved via the probe port kit) was used for online measurement and control of pH within the vessel[2]. The online control probe was not calibrated via 1-point offsets throughout the run, contrary to typical procedure. This was due to the desire to maintain reference probe data integrity throughout the test, with minimal impact on the cell culture. Throughout the long-term testing (day 21-60), the S.U.B. was subjected to adjustments in temperature with subsequent titrations performed in the culture broth to evaluate sensor for dynamic response and inaccuracies due to fouling. The OneFerm™ sensor readings were captured on Thermo Fisher AquaPro, 4 channel transmitters. Offline pH readings were performed approximately every 24 hours using a BioProfile FLEX II.

RESULTS

All 6 OneFerm™ sensors, aged for 24 months, met manufacturer's specifications after the cell run with no sensor exhibiting a gross pH drift greater than 0.29 during the entire 21 day period. Every sensor also met manufacturer's specifications during the 60 day hold period with no sensor exhibiting an average gross pH drift greater than 0.65 during this extended time. Furthermore, all sensors demonstrated an average response time of less than 20 seconds at neutral pH following the 60 day hold period.

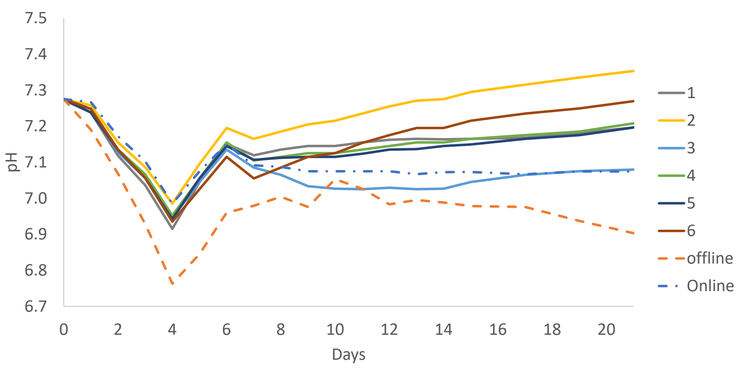

Figure 2. pH values averaged over a 30 minute window every 24 hours for the duration of the 21 day cell culture. The starting point was normalized.

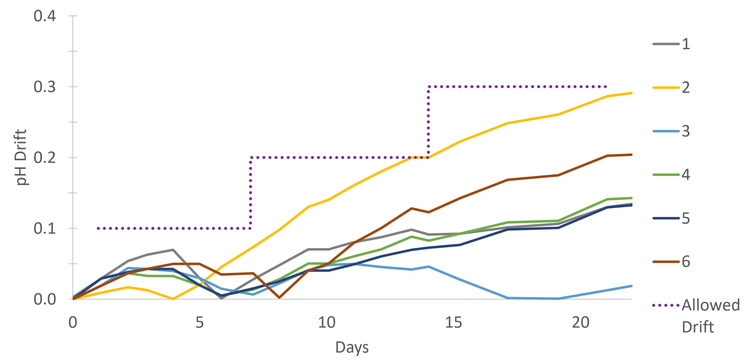

Figure 3. Overall drift of each sensor from the control probe throughout the 21 day culture, compared to the permissible drift.

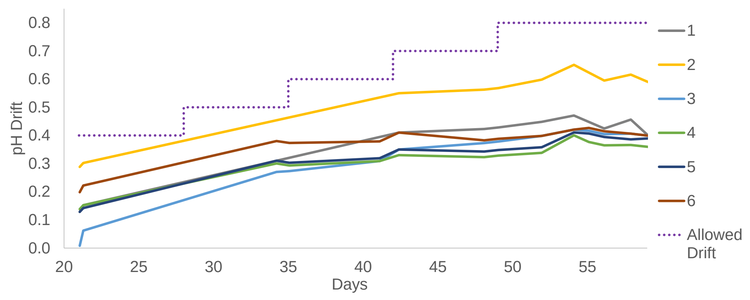

Figure 4. Long-term overall drift of each sensor from the control probe throughout the remaining 39 days of 60 days, compared to the permissible drift. Note: The data is discontinuous in nature due to titration steps, for simplicity only pH 7 data points are shown.

Table 1. Tabulated highlights from Figure 3 and 4. Showing best and worst case at 7, 14, 21, and 60 days.

Parameter	Supplier specification	Observation (best/worst)
Raw drift (7 day)	+/- 0.10 pH	+/- 0.01 pH / 0.07 pH
Raw drift (14 day)	+/- 0.20 pH	+/- 0.05 pH / 0.2 pH
Raw drift (21 day)	+/- 0.30 pH	+/- 0.05 pH / 0.29 pH
Raw drift (60 day)	+/- 0.80 pH	+/- 0.21 pH / 0.47 pH

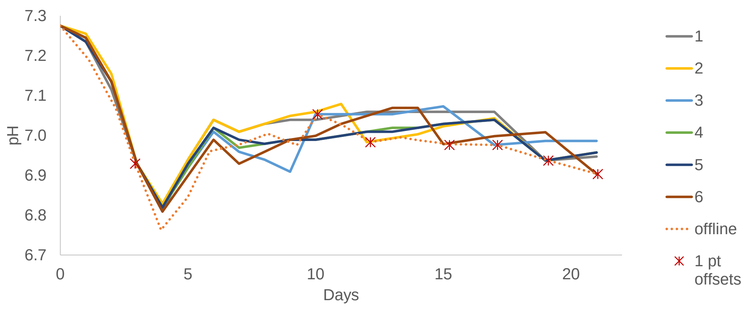

Figure 5. Calibrated pH data from each sensor throughout the 21 day culture. Each red mark indicates a 1-point offset to offline was applied to at least one sensor, in post-processing. This mimics real use where the sensor would be re-calibrated if it varied by more than 0.1 from offline. Worst case, sensor underwent 3 offsets over the 3 week period.

Recommendations and product specifications

- This data serves to verify the manufacturer qualification claim that the OneFerm™ pH sensor has 24 months of dry storage post-irradiation.
- During long-term post-gamma storage (more than two weeks), the gel in the reference electrode can pull away from the bulb due to gravity. For this reason, it is important that the BPCs be stored according to packaging labels to ensure proper performance.
- Operators and quality personnel should be aware that these sensors will inherently leach small amounts of KCl salt into the BPC. This particulate is a non-toxic salt originating from the reference electrode that may appear to be a foreign particulate during pre-use inspection.
- End users should take note: The BPC must be stored at the recommended temperature to ensure expected performance (4-40C).

Table 2. Product specifications and results

Requirement	Desired specification	As-tested results
Gamma stable	Tolerate > 25 kGy	30 kGy (+/- 3 kGy)
Biocompatible	USP class 6	Pass
Packaging/transport	ISTA 2A	Pass
BPC dry storage	Up to 18 months	24 Months: Pass
BPC integration	1 in. port	Pass, also 1 in. EJ port
pH range	3-10 pH	3-10 pH
Temperature range	5-37˚ C	2-50˚ C
Pressure range	> 5 psi	> 15 psi
Pre-calibration accuracy	0.1 pH	0.1 pH (0.05 pH w/ 1-point)
Low drift	< 0.1 per 7 days	Confirmed
Temperature comp.	Integrated	PT-1000, PT-100
Cable connector type	Keyed quick connect	VP-6 (twist lock)

CONCLUSIONS

The results of these experiments demonstrate the effectiveness of Hamilton OneFerm™ sensors in the Thermo Fisher Scientific single-use bioreactor, fermentor, and mixer platforms. Thermo Fisher Scientific has developed a custom polycarbonate probe port adapter to robustly integrate the OneFerm™ sensor in a BPC. The probes performed well after 24 months of dry-storage, post-irradiation. We are excited to share this growing body of data with the bioprocess industry as probe drift, ionic strength sensitivity, and shelf life have greatly limited implementation of SU pH over the past decade. These results appear to indicate a viable technology is available and is suitable for cGMP bio manufacturing.

REFERENCES

1.B. Madsen, 5:1 Culture in SUB 50L https://assets.thermofisher.com/TFS-Assets/BPD/Application-Notes/efficient-operation-evaluation-5-1-sub-working-volumes-app-note.pdf

2.A. Hodge, N. Jones, (2018) "Evaluation of the Hamilton™ OneFerm single-use pH sensor in the Single-Use Bioreactor" Thermo Fisher Scientific Technical Brief DOC0054

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