HAMILTON

CULTIVATED MEAT AND SEAFOOD

Innovative Solutions for Process Analytics

INTRODUCTION

By 2050, the annual global demand for meat is set to grow from 350 billion kg to 460-570 billion kg, while seafood is predicted to double to ~62 billion kg compared to today's consumption. This, along with environmental concerns including agricultural land availability and over-fishing highlights the need to find sustainable sources. Foods produced by cultivated methods could become staple components of future diets worldwide, however, developing this revolutionary technology is scientifically and culturally challenging. Critical hurdles include regulatory approval, developing a natural taste, scalability and price of the product - crucial for customer acceptance and feasibility of production.

PROCESS ANALYTICAL TECHNOLOGY

How to Achieve Commercially Viable Cultivated Meat

PAT in Process Optimization

To attain commercial scalability, the cost per unit of meat must undergo optimization. Within the industry, companies are presently placing a strong emphasis on research and development to determine the ideal parameters for their cultivated meat and fish production. Optimum cell growth depends on the process (fed batch, perfusion, continuous etc.), as well as the feeding strategy, pH conditions, and more. Regulating the sparge rate and mixing speed ensures favorable levels of dissolved oxygen (DO) and carbon dioxide (CO_2) for cell health and growth. Real-time monitoring of cell growth and better process insights are possible through cell density measurements. It is important to control these parameters to ensure process performance.

RECOMMENDED PRODUCTS: pH, DO, VCD, CO_2, TCD

PAT in Media Optimization

Arguably, the most expensive part of the cultivated meat and seafood process is media, which is necessary to sustain cellular growth in an artificial environment. According to the Good Food Institute, the costs of creating cell culture media will make up 55 to 95% of marginal costs of cultivated food production. Our advanced sensor suite, which includes pH, VCD, TCD, DO, DCO_2, and conductivity sensors, enables real-time monitoring of your process, empowering data-driven media formulation development. By utilizing real-time data and precise control, you can customize media composition to reduce costs, increase growth rates, maintain consistent high quality, and optimize yields.

RECOMMENDED PRODUCTS: pH, DO, VCD, CO_2, TCD, Cond

PAT in Scaling Strategies

Another significant challenge in producing cost-effective cultivated meat and seafood is scalability. Organizations are limited by the number of cells, and therefore the amount of cultivated product that can be created.

Successful application of scaling strategies (scale-up or scale-out) to generate large numbers of cells requires monitoring of processes, accounting for product demand at each stage of production, and optimizing conditions to improve process performance. Optimization is achievable through the implementation of control measures such as in-line process analytical technology (PAT), which offer real-time feedback on conditions in the bioreactor. Cells need sufficient supplies of oxygen and glucose, the correct pH, and the removal of carbon dioxide for efficient growth. Sensors tracking these key parameters in addition to total cell density and viable cell density - important indicators of cell health - ensure maximal yield.

RECOMMENDED PRODUCTS: pH, DO, VCD, CO_2, TCD

THE CULTIVATED MEAT AND SEAFOOD PROCESS

Cultivated Meat and Seafood processes rely on the growth of large volumes of cells either as the end-product themselves, or as synthesizers of biomolecules.

1. Stem cells and muscle cells harvested from target meat (initial focuses are chicken, beef, pork) and seafood (e.g., salmon, tuna, white fish, crab, lobster, shrimp) are multiplied through a seed train then introduced to the primary bioreactor. Bioreactors are typically stirred tanks or rocking platforms.
2. Once the primary bioreactor reaches optimum cell density, cellular differentiation is initiated through a combination of media components and adhesion to microcarriers and/or scaffolds. Differentiated cells mature into the final product through continued growth and adherence.

Where can in-line sensor systems be used during the cultivated meat process?

The main step where in-line sensors can help to improve process performance is at the primary bioreactor during proliferation.

- Critical Process Parameters (CPP) for all cell cultures = pH, DO, Dissolved CO_2, Temperature
- Key Performance Indicators (KPI) for all cell cultures = Cell Density (Total and Viable)

Secondary locations for in-line sensors are media prep tanks or perfusion systems providing media to the scaffold.

1 Cell Line Derivation

Starting cell line is created with stem cells and muscle cells harvested from live animals (without harming the animals). Cells are retrieved from embryos, biopsies, or iPSC.

2 Media & Water Prep

Media is prepared in media prep tanks, where pH and conductivity should be monitored. This ensures cell viability and enables optimal nutrient environment for cell growth. pH and conductivity should be measured in water prep tanks to verify purity before reverse osmosis. The **EasyFerm Bio pH Sensor** and the **Conducell 4USF Conductivity Sensor** are ideal choices for controlling these important parameters.

Cond pH

3 Bioprocess: Proliferation

Harvested cells are expanded through a seed train and introduced to the primary bioreactor. At this process stage the following parameters should be measured and controlled: pH, Dissolved Oxygen, Dissolved CO_2, and Cell Density. Ensuring ideal conditions for cells will promote optimized cell growth and maximize product yield and profitability. Hamilton's **Incyte Arc**, **Dencytee Arc**, **CO_2NTROL**, **EasyFerm Bio**, and **VisiFerm RS485** are optimal sensors for this step of the process.

TCD DO pH CO_2 VCD

4 Differentiation/Maturation

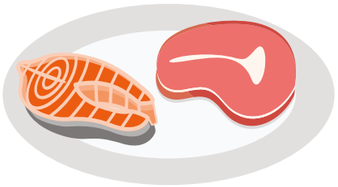
Once optimum cell density is reached, cells are encouraged to differentiate into the three main components of meat: muscle, fat, and connective tissue through a combination of media components (e.g., addition of growth factors facilitating myogenesis) and adhesion to microcarriers and/or scaffolds (depending on the cell type, process and structural complexity of the end product). Monitoring pH, Dissolved Oxygen, Dissolved CO_2, and Cell Density at this step help cells continue to grow and adhere. Controlling conditions allows cells to proliferate and to create the most product. Like during the proliferation phase, **Incyte Arc**, **Dencytee Arc**, **CO_2NTROL**, **EasyFerm Bio**, and **VisiFerm RS485** sensors enable optimal growth conditions.

TCD DO pH CO_2 VCD

5 Final Product

Cells have grown and adhered to a sufficient density to form the final product, which is ready for consumption. Measuring critical process parameters such as pH, Dissolved Oxygen, Dissolved CO_2, and Cell Density throughout the cultivated meat process enables better control and higher product yield.

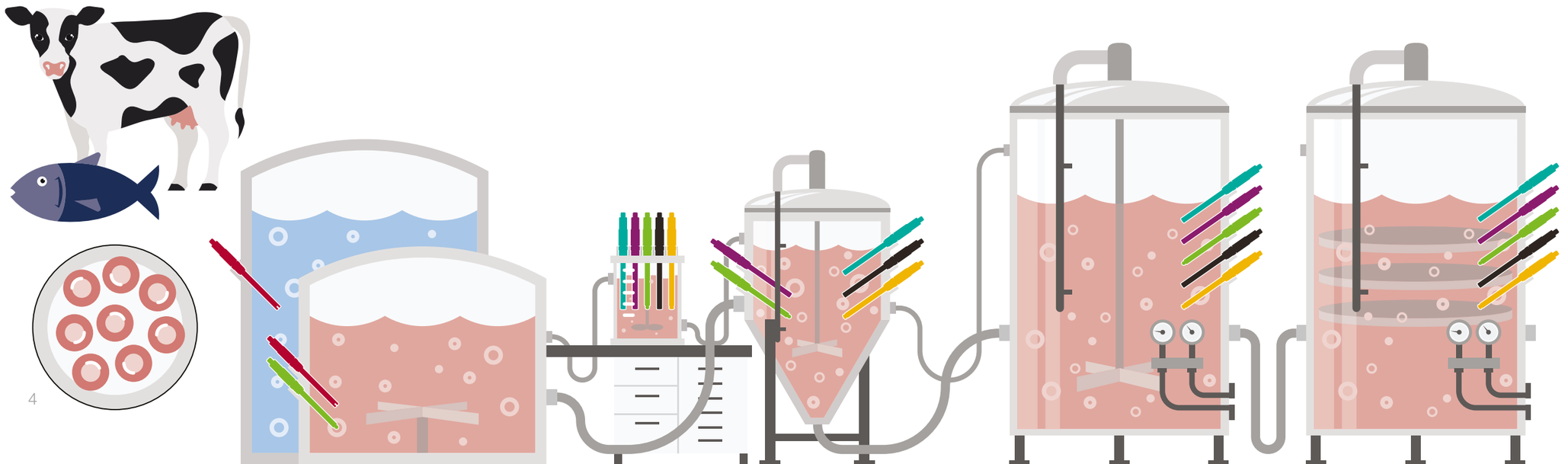
TCD DO pH CO_2 VCD



6 Media Reclamation & Water Treatment

Reusing media and water can be an option to reduce overall process costs. Correct pH levels must be maintained, and conductivity adjusted to provide ideal growth conditions. Wastewater treatment might require local pH levels to be met and therefore measured.

Cycle repeats back at step 2



MEET THE SENSORS

The Technology that Makes Cost Efficient Cultivated Meat and Seafood Production Possible

Hamilton's state-of-the-art technology empowers organizations to achieve complete control over their bioprocess, resulting in reduced production costs, seamless automation, and successful scale-up for commercialization.

Hamilton understands the critical importance of maintaining an optimal environment for cell culture. Our sensors are designed to provide precise and real-time feedback, ensuring a level of efficiency that boosts productivity while significantly reducing production expenses, making your cultivated food endeavors economically sustainable.

DID YOU KNOW?

Hamilton also offers Single-Use Sensor options for pH and conductivity measurement for media preparation and process control.

Viable Cell Density Sensor

Hamilton's Incyte Arc Viable Cell Density sensor provides production with direct measurements of produced meat mass. Regardless of changes in media, dead cells, microcarriers, or debris, the sensor provides real-time accurate metrics, providing organizations with more control over processes for higher yields, lower operating costs, and improved reproducibility.

Total Cell Density Sensor

Total Cell Density is a KPI for cell growth that is applicable in most cell-culture workflows from R&D to large-scale production. By measuring turbidity, it provides information on the total cell content of cultures, however, is unable to distinguish between living and dead cells. Hamilton's Dencytee Arc enables real-time, in-situ measurement of total cell density.

Dissolved CO_2 Sensor

To create high-quality cultivated meat, CO_2 levels must remain within an appropriate range, and studies have shown that total removal of dissolved CO_2 is not necessarily the best strategy. Therefore maintaining dissolved CO_2 within the optimum range is key for cell growth. The CO_2NTROL dissolved CO_2 sensor continuously collects real-time measurements of dissolved CO_2 for live feedback on processes, and can be used to regulate dissolved CO_2 at levels that can improve process yields.

pH Sensor

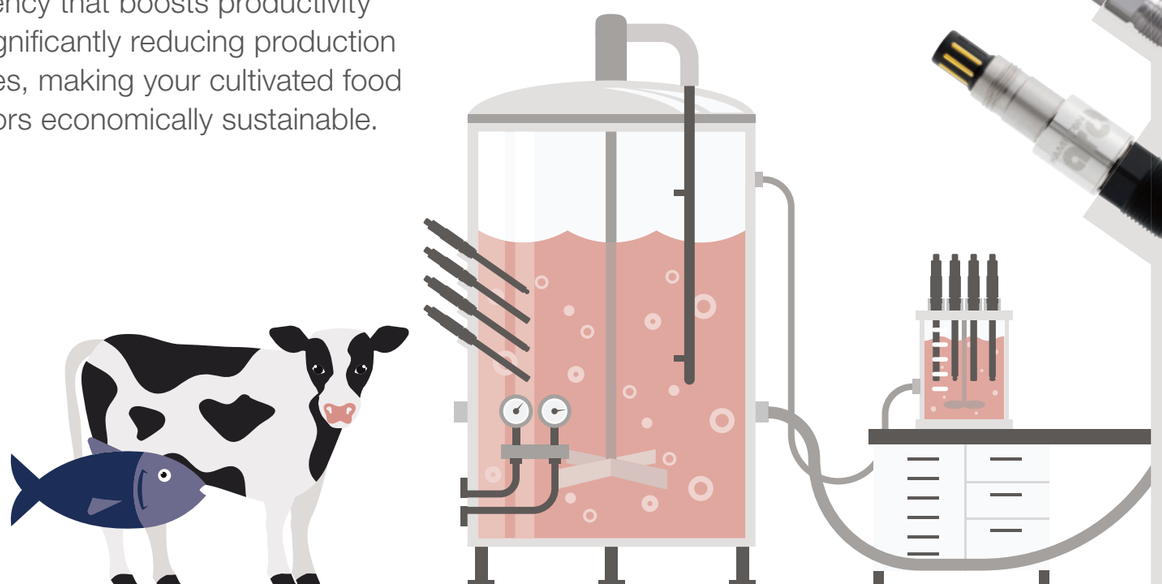
Measurement and precise control of pH levels throughout the cultivation process can drastically speed up cell growth. Coupled with the biocompatible Foodlyte electrolyte, the EasyFerm Bio pH Sensor ensures high-quality food production while keeping costs low.

Dissolved Oxygen Sensor

One of the most important factors for cellular respiration and cellular growth is oxygen. The VisiFerm RS485 Sensor speeds up cell growth and increases product output by measuring DO in-line for tighter process control.

Conductivity Sensor

Hamilton's Conducell 4USF Conductivity Sensor is the ideal sensor to measure conductivity in water treatment and media preparation tanks in cultivated food processes. Producing your own media saves the cost of expensive pre-made solutions, while recycling used media can further improve the cost economics of media usage during production. Conductivity is essential for ensuring water purity both during media production and recycling of water at the end of a production cycle.

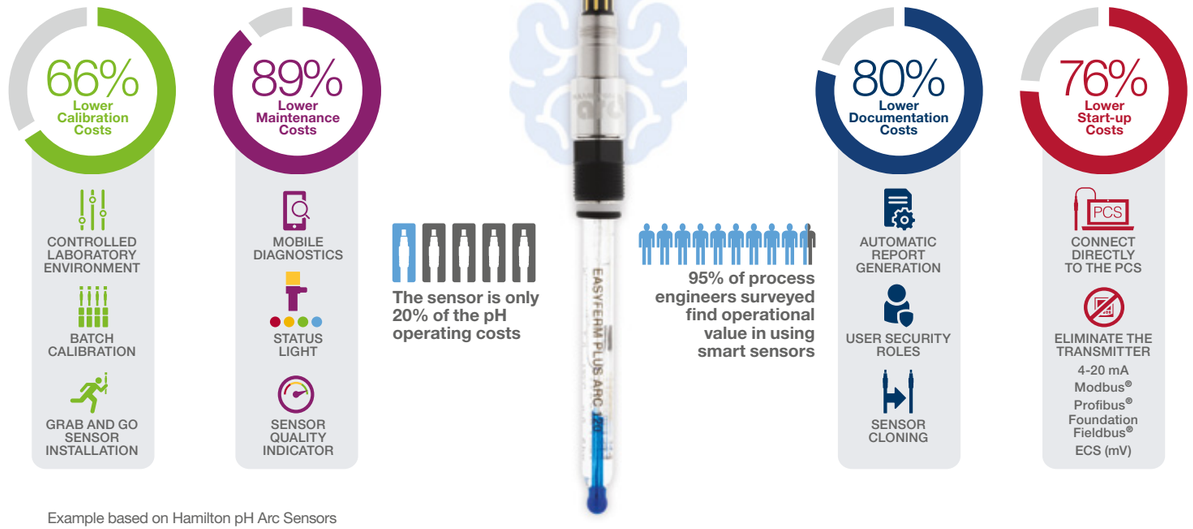


THE INTELLIGENT SENSOR REVOLUTION

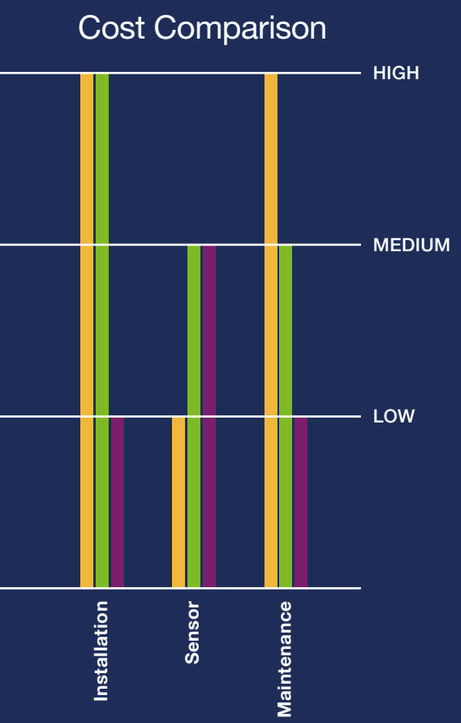
Over 95% of process engineers surveyed find value in using intelligent sensors

Less than half of engineers felt they fully utilized their smart sensor. The key to getting the most out of your smart sensor is choosing the implementation that best fits your application. Hamilton offers two versions of smart sensors each equipped with our best-in-class measurement technology.

Benefits of Intelligent Sensors



Example based on Hamilton pH Arc Sensors



▶ Analog Sensors

Traditional analog sensors offer the lowest sensor cost which is ideal for implementations that discard the sensor after each run. They rely on a transmitter to read the sensor's electrochemical signal (ECS) and transmit it to the process control system.

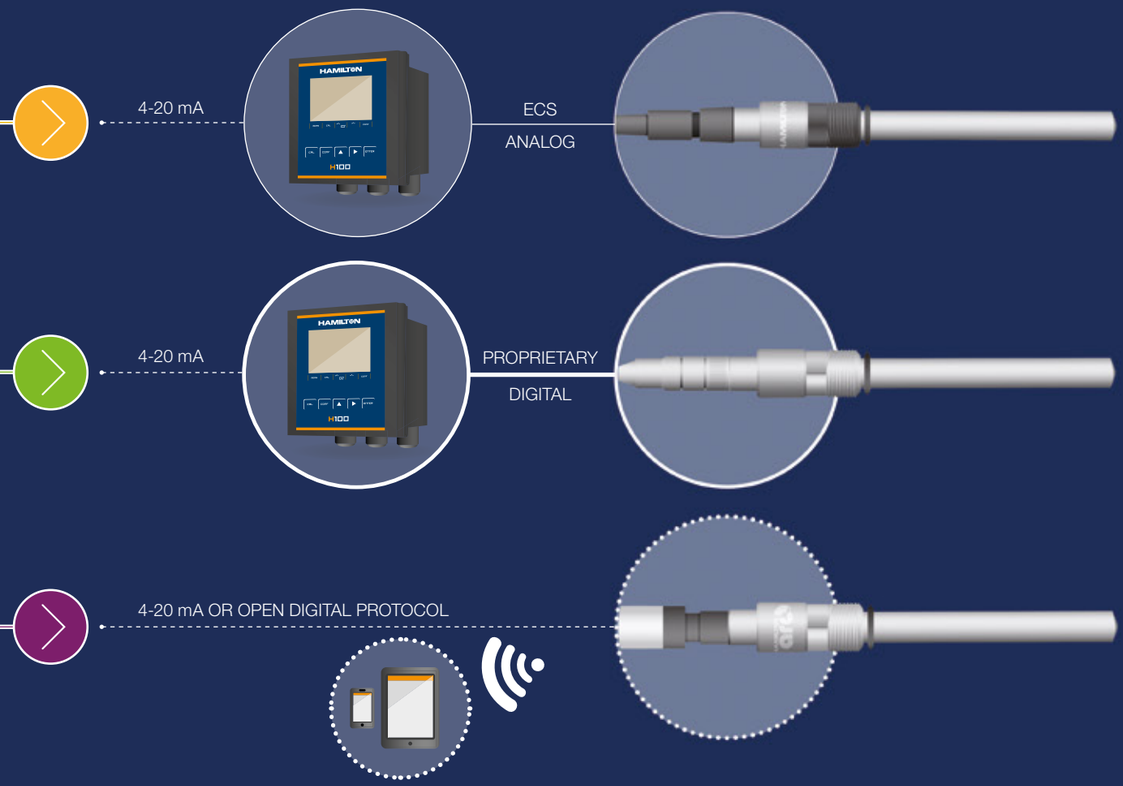
▶ Digital Sensors

Memosens

Digital sensors eliminate signal errors from electromechanical noise near the cable and reduce maintenance costs by enabling in-lab calibration instead of at-line. This is accomplished with an integrated processor to convert the electrochemical signal to a digital output.

▶ Arc Intelligent Sensors

Intelligent sensors have the lowest start-up and operating costs. They provide advanced alarms, troubleshooting, quality indicators, and diagnostics simultaneously to the control system and an optional mobile device. An integrated micro-transmitter eliminates a point of failure by directly connecting the sensor to the PCS.



HAMILTON SENSORS DETAILS AND BENEFITS

VisiFerm Family

The VisiFerm is the optimal optical process sensor for the measurement of dissolved oxygen in cultured food applications and processes. It has significant advantages compared to classical Clark cells. Available with the innovative Arc technology.

BENEFITS

- Self-diagnostics
- Electrolyte-free
- No polarization time required
- Easily replaceable sensor cap
- Digital and analog (4—20 mA) signal output
- ECS-mode available (Clark cell simulation)
- Robust design for steam sterilization, autoclavation, and CIP

EasyFerm Bio

The EasyFerm Bio families of pH and ORP sensors are ideal for biotech and cultivated food applications, like fermentations, where autoclavations, steam sterilizations (SIP), and cleaning in place (CIP) procedures occur frequently. Available with the innovative Arc technology.

BENEFITS

- Stable measurement signals after steam sterilization, autoclaving, and CIP
- Drift-free measurement
- Ceramic diaphragm
- Wetted parts according to USP class VI
- Available as ORP sensor
- EasyFerm Bio is bio-compatible (USP 31, 2008, chapter 87)

*Memosens

Incyte Arc

The Incyte sensors enable realtime, on-line measurement of viable cells in cultivated food, yeast, and fermentation processes. On-line measurement allows the detection of events and responses in real-time without sampling.

BENEFITS

- Insensitive to media changes, microcarriers, dead cells
- Detect changes in physiology with frequency scanning
- Early detection of deviations without sampling
- Increase yield and lower production costs

Dencytee Arc

The Dencytee Arc sensors perform on-line measurement of total cell density based on optical density at NIR (near infrared) wavelengths. Thanks to its combined measuring principle of transmission and reflection measurement, Dencytee Arc delivers reliable TCD values over the complete process at low and high cell concentrations.

BENEFITS

- Simple on-line measurement of cell growth
- Reliable values during the growth phase
- Improved linearity at high concentrations
- Early detection of process deviations

CO_2NTROL

The CO_2NTROL sensor performs online measurement of dissolved carbon dioxide using MIR (mid-infrared) wavelengths.

BENEFITS

- Direct quantification of dissolved CO_2
- Solid-state sensor requiring minimal maintenance
- Enables real-time CO_2 control for optimizing product yield
- EHEDG certified

Conducell 4UxF

The Conducell 4UxF family of 4-pole conductivity sensors is suitable for measuring a broad range of conductivities with excellent linearity. Available with the innovative Arc technology.

BENEFITS

- Robust design for steam sterilization, auto clavation, and CIP
- All wetted parts are FDA-compliant
- Very easy to clean due to the forward-facing, flush arrangement of electrodes
- EHEDG-certified with Hamilton's hygienic socket
- Available pole materials: stainless steel, Hastelloy C, and titanium
- Various process connections available: PG 13, 5, Tuchenhagen VariVent, BioConnect, and Triclamp

To find a subsidiary or distributor in your area, please visit,
www.hamiltoncompany.com/process-analytics/support.

SCAN TO READ OUR APP NOTE

Potential to Maximize Cultivated Meat Productivity Through VCD In-Situ Measurement

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