



● **Grow *Campylobacter* and Similar Bacteria Using Less Oxygen**

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Introduction

- Why culture bacteria using less oxygen?
 - *In vivo*, especially in the GI tract, oxygen concentrations are much lower than in the air we breathe
 - Optimum culturing requires mimicking native environment
 - Many sources now recommend that *Campylobacter* and other microaerophilic bacteria be cultured in a “microaerobic” atmosphere
 - USDA, March 2011
 - European Union, 2007
 - *Salmonella* species are up to 70% more invasive when cultured under very low oxygen

Lee and Falkow *PNAS* 1990

Campylobacter Incidence in United States

- *Campylobacter* species have been identified as the second most common cause of foodborne illness in the U.S. behind *Salmonella*
- 2011 CDC report estimates 845,024 cases of campylobacteriosis each year

U.S. Centers for Disease Control and Prevention. National Center for Emerging and Zoonotic Infectious Diseases: Campylobacter 2011.



Campylobacter Incidence in European Union

- Number one cause of foodborne diarrhea in Europe
- In 2005, EU reported 194,695 cases of illness caused by *Campylobacter* in 22 Member States

The EFSA Journal 94:2006.

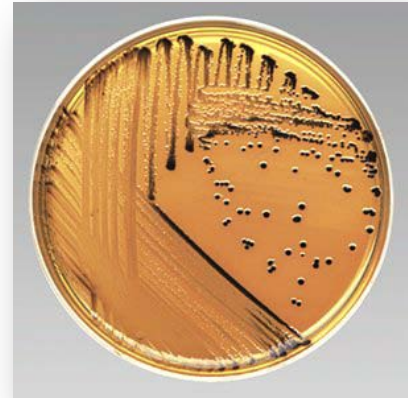


<http://europa.eu/abc/maps/>

Salmonella Incidence in United States

- *Salmonella* species are the number one cause of foodborne illness in the U.S. each year
- 1,027,561 estimated cases each year

U.S. Centers for Disease Control and Prevention. National Center for Emerging and Zoonotic Infectious Diseases: Campylobacter 2011.



Salmonella Incidence in the European Union

- Number two cause of foodborne illness in EU
- 168,929 cases of *Salmonella* infection in humans reported in 22 EU Member States in 2005

The EFSA Journal 94:2006.

- *Campylobacter* is a major cause of diarrhea in infants

- Found in about half of all puppies

Brock and Madigan, *Biology of Microorganisms* 5th Ed. 1988



Wikimedia Commons

Why Culture *Campylobacter* Under Low Oxygen?

- Recent research demonstrates that growing *Campylobacter* and similar bacteria under low oxygen conditions (“hypoxia”) significantly improves recovery and growth
 - Which better enables successful diagnosis and treatment

“Use of oxygen-quenching agents, a microaerobic atmosphere, and antibiotics that suppress competitors, significantly improve *Campylobacter* recovery.”

United States Department of Agriculture, Food Safety Inspection Service (FSIS), Office of Public Health Science (OPHS) Risk Assessment Division. Potential public health impact of *Salmonella* and *Campylobacter* performance guidance for young chickens and turkeys. January 2011.



Low Oxygen Culture Will Save Millions of Dollars

- Testing methods established in 2011 for *Campylobacter* and *Salmonella* are predicted to prevent 30,000 cases of foodborne illness in the U.S. after implementation

United States Department of Agriculture, Food Safety Inspection Service (FSIS), Office of Public Health Science (OPHS) Risk Assessment Division. Potential public health impact of *Salmonella* and *Campylobacter* performance guidance for young chickens and turkeys. January 2011.

- This equals approximately \$81 million EACH YEAR saved in costs of illness

Calculation based on data from Batz, MB, Hoffmann S and Morris JG Jr. Ranking the Risks: The 10 Pathogen-Food Combinations With the Greatest Burden on Public Health. University of Florida, 2011.



How to Obtain Low Oxygen Culture?

- EU recommends:

- “The micro-aerobic atmosphere may be obtained in commercially available micro-aerobic incubators (gas mixture 10% CO₂, 6% O₂)....(or other) culture systems can be used i.e. gas jars.”

Official Journal of the European Union, Commission Decision of 19 July 2007, LL 190/25

- USDA recommends:

1. “42 +/- 1°C Tri-gas incubator (static) charged with 5% O₂, 10% CO₂, and 85% N₂”
2. “Gas cylinders containing appropriate gas mixtures to achieve 5% O₂, 10% CO₂, and 85% N₂ with regulators compatible with Compressed Gas Association (CGA) connection on the cylinder.”
3. “Commercially available gas packs intended for *Campylobacter* testing.”
4. “Bags or other containers capable of maintaining the atmosphere during incubation.”

United States Department of Agriculture, Food Safety Inspection Service (FSIS), Office of Public Health Science (OPHS) Risk Assessment Division. Potential public health impact of *Salmonella* and *Campylobacter* performance guidance for young chickens and turkeys. January 2011.

Microaerobic Culturing Options

- Anaerobe jars and gas packs



- Tri-gas Incubator



- Modular sealed chamber

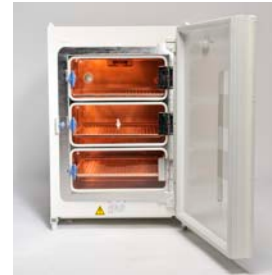


Fig. 05 | Sealed Modular Incubator Chamber



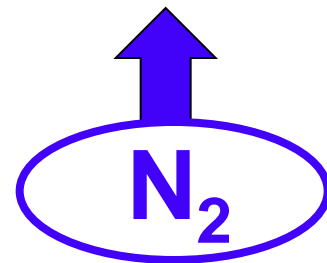
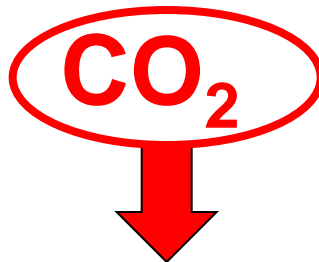
Tri-Gas Incubators: How They Work

- Tri-Gas incubators reduce oxygen concentration by pumping in nitrogen gas
- Sensor measure oxygen concentration and trigger N₂
 - Fuel cell
 - Zirconium oxide
- Oxygen concentration is set via control panel, like for CO₂
 - Use with CO₂ only for standard incubator



Thermo Fisher Scientific Leads with Tri-Gas Incubators

- Introduced the tri-gas incubator in 1979
 - Only two years after Packer and Fuehr recommended lower oxygen for cultured cells in 1977
 - FDA 510(k) registered for use with human patient samples
- Adding in nitrogen gas is technically tricky
 - CO₂ gas sinks
 - N₂ gas rises
 - Humidity, temperature and CO₂ affected



➔ Active air circulation is the only way to keep conditions uniform throughout the chamber!

Summary and Conclusions

- *Campylobacter* species are “on the rise” as foodborne pathogens, causing thousands of illnesses each year
- USDA and EU recommend culturing *Campylobacter* samples in a microaerobic atmosphere
- Low oxygen culturing of *Campylobacter* and *Salmonella* species alone is predicted to save thousands of illnesses and millions of dollars each year
- Microaerobic conditions are most easily and reliably obtained using a “tri-gas” incubator
 - Easy to use
 - No risk of losing samples due to gas leakage

Technical Resources

The screenshot shows the Thermo Scientific website's product page for CO2 incubators. The header includes the Thermo Scientific logo, a user account section, and navigation menus for Home, Products, Applications, Services & Support, News, Events, and Contact Us. A search bar is visible. The main content area features the heading "Thermo Scientific CO₂ Incubators" and a sub-heading "Leave your worries at the door!". Below this, there is a paragraph describing the benefits of their incubators and a "Download free brochure" button. A sidebar on the right offers "Need Help?" options, including "Have a Question?", "Visit us here to help you", and "Contact Sales".

thermoscientific.com/co2incubators

The cover of Lab Manager magazine features the headline "CELL CULTURE CONTAMINATION - PART 2". The sub-headline reads "UNDERSTANDING THE CAUSES AND MANAGING THE RISKS". The cover also includes the text "Contamination in what you do endangers the life of cell culture in reliable reagents and tools."

This page from Lab Manager magazine contains an article titled "Culturing Cells Under Hypoxic Conditions for Biologically Relevant Results". The article discusses the importance of hypoxic conditions in cell culture and provides practical advice for researchers.

The "smart notes" article is titled "Why is a consulting firm essential to superior growth conditions in a CO₂ cell culture incubator?". It provides expert advice on optimizing cell culture environments for better results.

The "Laboratory Equipment" article is titled "Oxygen: Too Much of a Good Thing". It discusses the impact of oxygen levels on cell culture and offers solutions for maintaining optimal conditions.

A collage of technical articles and brochures. One article is titled "Importance of Class 1 in a CO₂ Incubator". Another article discusses "Decontamination Cycles in Heraeus BBD 6220 and Heraeus Incubators Completely Eliminate Mycoplasma". A third article is titled "MAINTAINING A CLEAN CELL CULTURE ENVIRONMENT". The collage also includes a graphic with the text "5 reasons why your next CO₂ incubator should be 100% pure copper" and the Thermo Scientific logo.