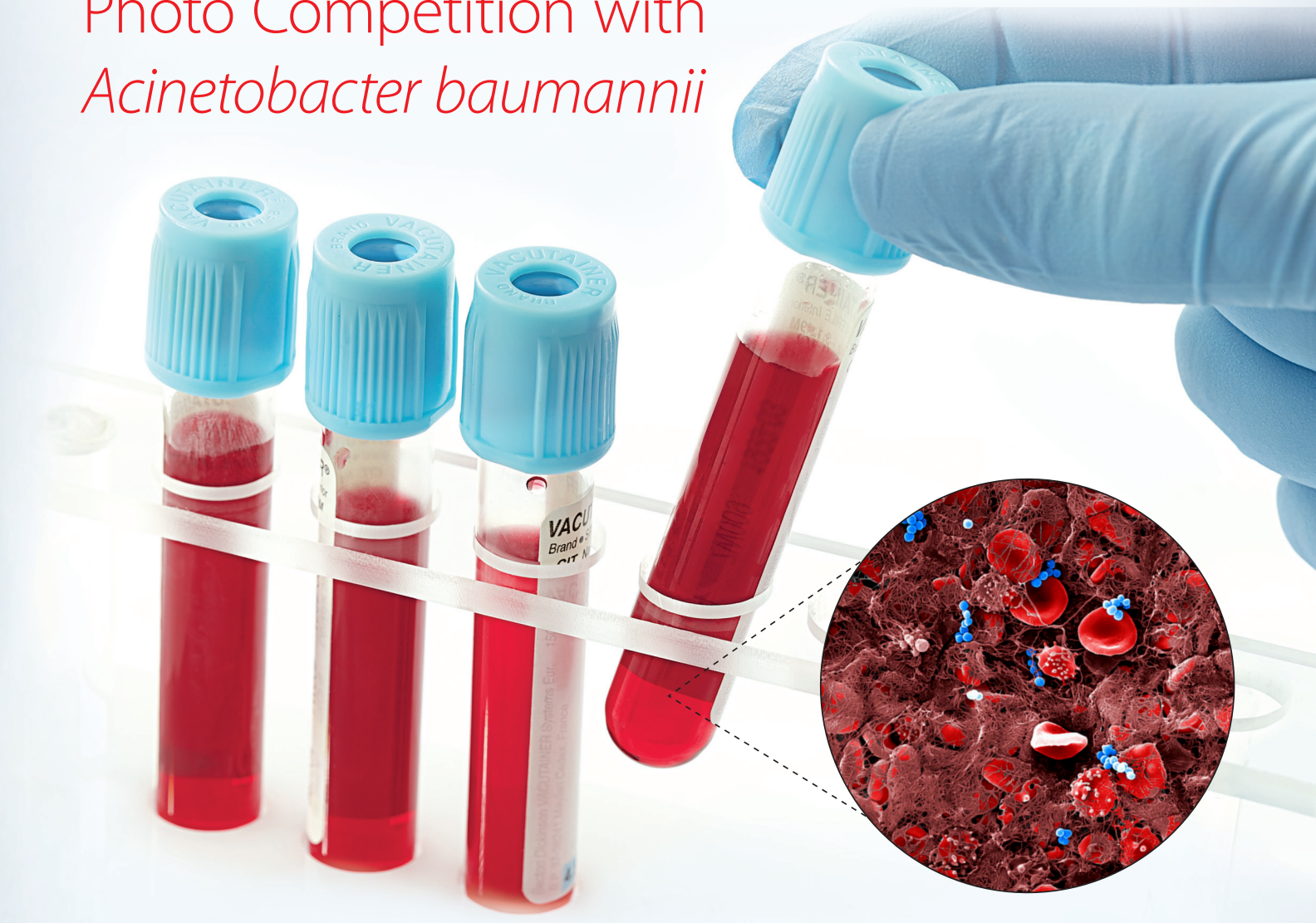
Volume 8.2 • 2016

Microbiology Focus

Winner of the Photo Competition with *Acinetobacter baumannii*



Acinetobacter baumannii is a potential hospital–acquired and multi–resistant pathogen. The winning photo in our recent competition is of a microscopic image where *Acinetobacter baumannii* (in blue) is growing between fibrinogen fibers and erythrocytes (red cells) in a clot of human blood.

Winners of the Photo Competition

By Jvo Siegrist, Product Manager Microbiology
ivo.siegrist@sial.com

Compared to chemistry or physics, microbiology's visible and impressive images can demonstrate how interesting and manifold microbiology truly is.

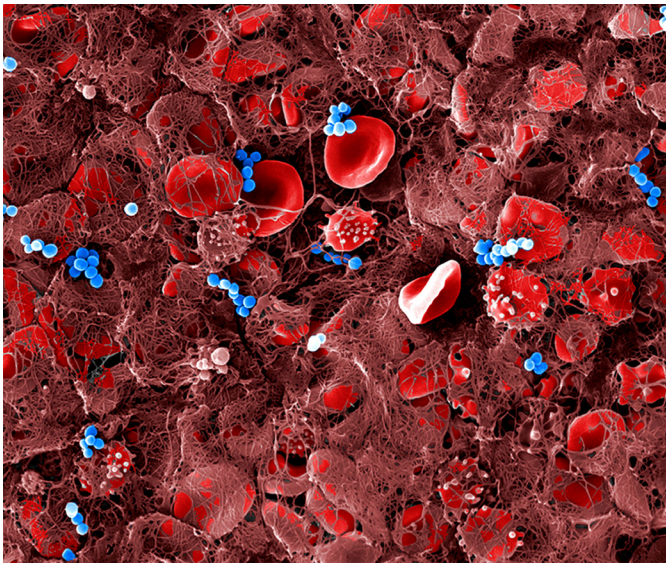
Front Cover

The interesting and intriguing microscopic image on the front cover of this issue of *Microbiology Focus* is the winning photo from the 2015 Sigma-Aldrich® Microbiology Photo Competition. It captures *Acinetobacter baumannii* (in blue), a Gram-negative pathogen which is a cause of hospital-acquired infections, in a clot of human blood. *Acinetobacter baumannii* is growing between fibrinogen fibers and erythrocytes (red cells). This is a dangerous situation for a bedridden patient. Read more about *Acinetobacter baumanii* in the special article.

Several microbiologists availed themselves of the opportunity to promote both their work and their institute, with fascinating photographs of microorganisms. We received numerous entries, and the decision was not an easy one for the jury. We thank all of the microbiologists who sent us their splendid images, along with their interesting and amusing descriptions. We would also like to thank our independent jury members Prof. Dr. Lars Fieseler (Zurich University of Applied Sciences) and Prof. Dr. Mohammad Manafi (Medical University of Vienna). The five winners received prizes which included an Android™ tablet PC, a Sigma-Aldrich® Giant Microbe, a Swiss army knife and a USB stick. We hope our winners enjoy their prizes as much as we have enjoyed hosting the photo competition!

View the images from all attendees on our website:
sigma-aldrich.com/microbiology

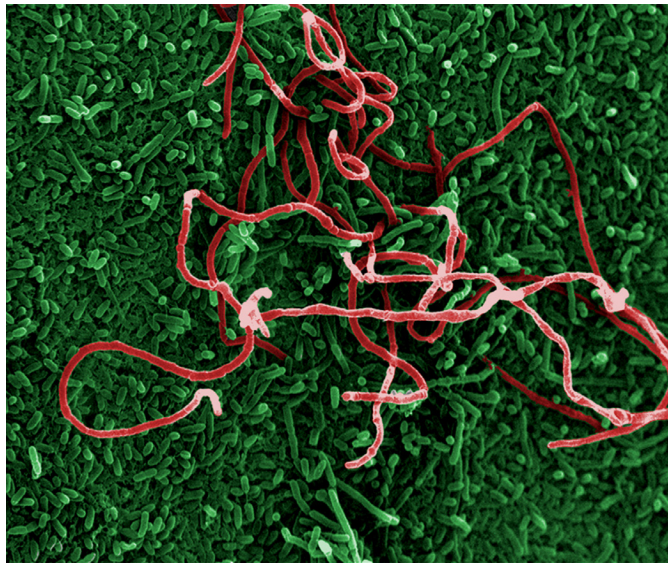
1st Place: José Ramos Vivas (IDIVAL Research Institute)



Blood Clot Infection

The Gram-negative pathogen *Acinetobacter baumannii* has become an increasingly prevalent cause of hospital-acquired infections during the last two decades, primarily resulting in pneumonia and complicated infections including wound infections in troops injured in Afghanistan and Iraq. The scanning electron micrograph shows a clot of human blood infected with *Acinetobacter baumannii* (blue). Fibrinogen fibers with entrapped erythrocytes (red cells) form the thrombus, or blood clot. Original magnification ×5,000.

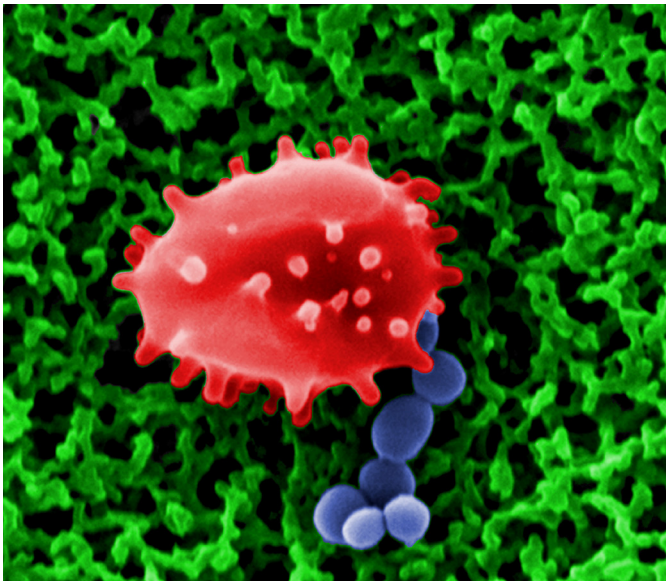
2nd Place: José Ramos Vivas (IDIVAL Research Institute)



Pseudomonas Snake

The formation of the organized bacterial community called biofilm is a crucial event in bacterial physiology. The scanning electron micrograph shows a long bacterial chain (red) emerging from a *Pseudomonas aeruginosa* biofilm (green). Cellular chaining may facilitate an increased flow of nutrients through the interior of the biofilm and could also afford an increased structural coherence during stress conditions. Original magnification ×5,000.

3rd Place: José Ramos Vivas (IDIVAL Research Institute)



Blood Sea Urchin Being Infected

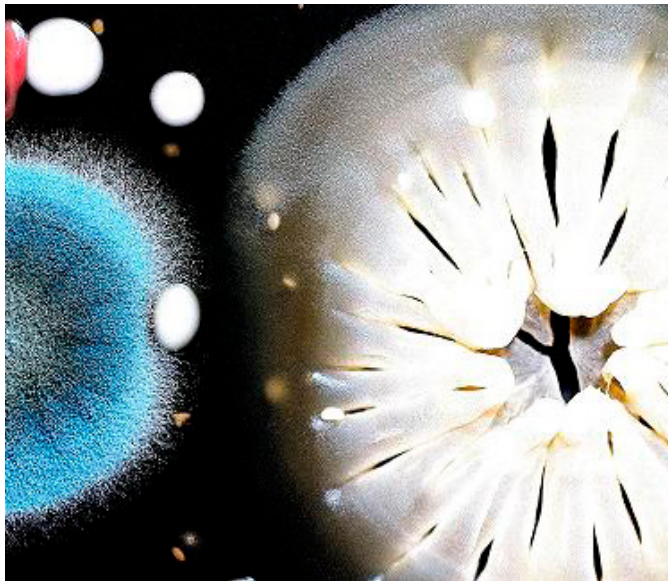
Adherence to host cells is the first step in bacterial colonization/infection. The scanning electron micrograph shows a human echinocyte (red) in close contact with the pathogen *Acinetobacter baumannii* (purple). Echinocyte (from the Greek word "echinos," meaning "sea urchin"), refers to a form of red blood cell that has an abnormal cell membrane. Original magnification ×12,000.

4th Place: Joefrhym Merillana (University of Southern Mindanao)

Invasion

Fungal contamination.

5th Place: Stefania Rizzelli (Artist)



The Invisible Art of Bacteria

The project "The Invisible Art" has the intention of promoting the works of art created by some bacteria grown in a Petri dish. Following their natural instincts they multiply, creating a range of forms and types of colonies. They are defined as the first true artists of the Earth.

Artist Stefania Rizzelli combines food and art materialized in a series of artistic photographs demonstrating the existence of natural and silent art created by bacteria in food, visible only with specific laboratory techniques.

Did you know …

Acinetobacter baumannii is easily spread in hospitals?

A study found that approximately 40% of protective gowns and gloves worn by healthcare workers who were exposed to patients with multidrug-resistant *Acinetobacter baumannii* became contaminated during contact. (Morgan et al. 2010.)

Figure 1: Gowns and gloves used in a hospital

Acinetobacter as a Pathogen

By Jvo Siegrist, Product Manager Microbiology
ivo.siegrist@sial.com

Over the last few years *Acinetobacter* has been attracting attention, especially because of its multi resistant strains.

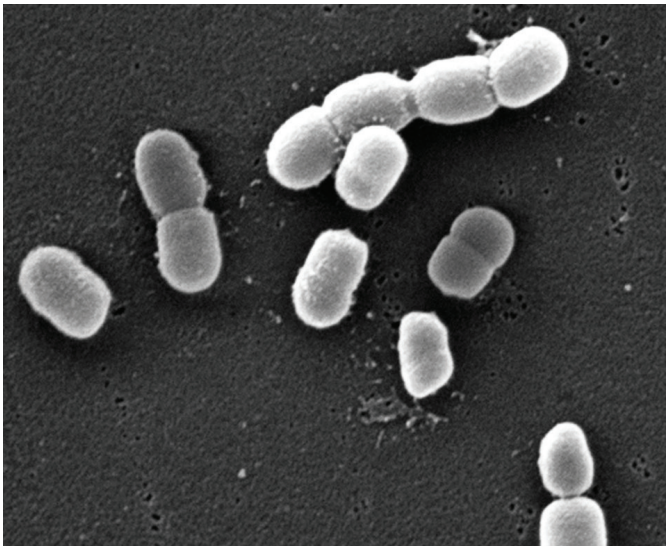
The most common species involved in clinical infections is *Acinetobacter baumanni*, which can cause pneumonia, wound infections and meningitis (although in most cases, *A. lwoffii* is responsible for meningitis.) Overall, 20 strains are known, but *A. baumanni* is primarily related to multi-resistance.

Acinetobacters are Gram-negative, strictly aerobic and non-motile. They count to the bacilli, but in most cases they show a coccobacillary morphology on nonselective agar and rods in liquid media. The name comes from the Greek "akínetos" which means "non-motile" and "bakterion" which means "rod".

Biochemically they are non-fermentative, oxidase negative and usually nitrate negative. Often they are partially lactose fermenting on MacConkey agar, although officially, they are classified as lactose negative. In the absence of elements such as phosphorus, nitrogen, or oxygen combined with a carbon source surplus, *Acinetobacter* is able to produce polyhydroxyalkanoates, a bioplastic. This is a form of intracellular storage substance which is built into so-called inclusion bodies.

Classification	
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Pseudomonadales
Family	Moraxellaceae
Genus	*Acinetobacter*

Figure 2: *Acinetobacters* showing Coccobacillary Appearance (Source Jose Ramos Vivas, IDIVAL Research Institute)



Acinetobacter can be identified by grow morphology on blood agar (incubation 24 hours), colonies with 0.5–2 mm diameter, translucent to opaque (never pigmented), convex and entire. Most strains grow well on MacConkey agar, showing a faint pink tint. A presumptive identification is the lack of cytochrome oxidase, non-motile and the resistance to penicillin.

Acinetobacter baumanni can be differentiated by observing acid production from glucose by growth at 41 °C and 44 °C, production of β-xylosidase and usage of malate.[1] To get selectivity to multi resistant *Acinetobacter*, the antibiotic imipenem, a carbapenem, can be added.[2,3]

Table 1: Typical media for *Acinetobacter*

Medium	Cat No.
Blood Agar (Base)	70133
Blood Agar Base No. 2	B1676
Brain Heart Infusion Agar	70138
Brain Heart Infusion Broth	53286
Brain Heart Infusion Broth (Sachets)	75189
Columbia Agar	27688
MacConkey Agar	M7408
MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15% Bile Salts	M8302
MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15% Bile Salts (ready-to-use media in the bottle)	94216
MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15% Bile Salts (Sachets)	92937
MacConkey Broth	70144
MacConkey Broth Purple	16377
MacConkey-Agar (without salt)	51405
MacConkey-Sorbitol Agar	88902
OF Test Nutrient Agar	75315

Table 2: *Acinetobacter baumannii* as Certified Reference Material for Performance Testing, Validation or Research

Species	Origin	Strain No.	CFU
Acinetobacter baumannii	ATCC®	19606™	80
Acinetobacter baumannii	ATCC®	19606™	200
Acinetobacter baumannii	ATCC®	19606™	1,000

References

1. Loneman, E. W.;. Allen, S. D.; Janda, W. M.; Schreckenberger, P. C.; Winn, Jr., W. C. Color Atlas and Textbook of Diagnostic Microbiology, 5th edition; Lippincott, Williams & Wilkins, **1997**.

Lautenbach, E.; Synnestvedt, M.; Weiner, M. G.; Bilker, W. B.; Vo, L.; Schein, J.; Kim, M. Epidemiology and Impact of Imipenem Resistance in Acinetobacter baumannii. *Infect Control Hosp Epidemiol* **2009**, 1186–92.

2. Manchanda, V.; Sanchaita, S.; Singh, N. P. Multidrug Resistant Acinetobacter, *J. Glob. Infect. Dis.* **2010**, *2* (3), 291–304.

Rapid Immunodetection of *Campylobacter*

By Diana Spitznagel, Ph.D., Global Product Manager, Merck Life Science GmbH, Eppelheim
diana.spitznagel@merckgroup.com

There is an increasing demand for awareness of contamination levels regarding *Campylobacter* in poultry. Testing methods should provide optimal growth conditions and, at the same time, be cost-efficient. An innovative immunological method enables earlier detection of contamination at poultry farms and processing plants.

It is estimated that one in three people in industrialized countries may be affected by foodborne illnesses each year.[1] According to the European Food Safety Authority (EFSA), campylobacteriosis, caused by the pathogen *Campylobacter*, is the most frequently reported cause of food-related illness in the European Union, with an estimated nine million cases each year.[2] The EFSA has calculated the cost of campylobacteriosis to public health systems and lost productivity in the EU to be around EUR 2.4 billion per year. In the United States, the Centers for Disease Control estimates that campylobacteriosis affects over 1.3 million persons every year.[3]

There is an increasing demand for *Campylobacter* testing of food, and a new awareness of contamination levels regarding *Campylobacter* in poultry. To protect consumers from this public health threat, regulations and policies are being implemented to shift the focus from contamination response to prevention.

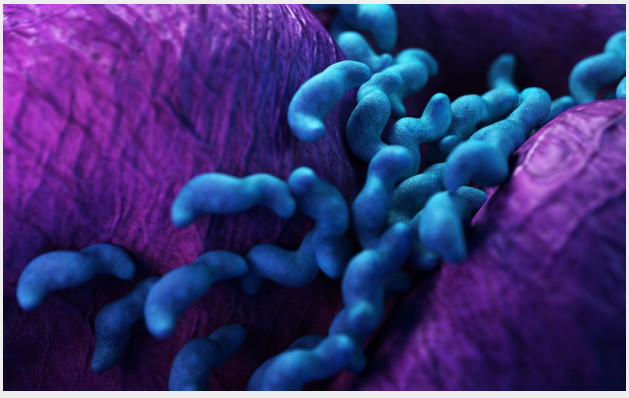
The EU has adopted an integrated approach to food safety from the "farm to the fork." The approach consists of both risk assessment and risk management, measured at different stages of the food chain system, and examines the practices and procedures that ensure food safety. Similarly, the U.S. has seen a sweeping reform of its food safety laws with the Food Safety Modernization Act of 2011.

Did you know …

C. jejuni lacks many of the common metabolic pathways?

Usage of glucose, galactose, or other carbohydrates is not possible. It does, however, make efficient use of citric acid cycle intermediates and various amino acids.

Figure 1: Gowns and gloves used in a hospital



The act represents a paradigm shift to prevention by establishing a modern system of food safety protection based not on reacting to problems but rather on preventing them from happening in the first place.

Initiatives targeting *Campylobacter* are similar to those instituted in recent years to better prevent *Salmonella* contamination. Coordinated approaches for controlling *Salmonella* in poultry have been hailed as a success, and it is hoped that efforts focused on *Campylobacter* will deliver equivalent results. *Salmonella* cases continue to decline in EU member states, and most member states met their reduction goals for prevalence in poultry in 2013.[4]

The *Campylobacter* Challenge

Campylobacter can be introduced to poultry flocks in a number of ways. Contaminated feed, insects or bacteria from human interaction can introduce the pathogen, causing the *Campylobacter* status of an entire flock to change from negative to positive within a few days.

To identify risk of contamination, it is critical to screen live chickens for *Campylobacter*. This enables segregation of contaminated flocks at the farm, ahead of slaughter. While testing methods do exist, standard microbiological testing and real-time PCR require samples to be analyzed in a laboratory setting by trained personnel, not directly on the farm, and can take up to several days to obtain results. Since the *Campylobacter* status of an entire flock can change so quickly, such results may be of limited predictive value. For the most up-to-date information, testing for *Campylobacter* in poultry should be done as close as possible to slaughter.

During the slaughter, plucking and evisceration can lead to contamination of carcasses; if a *Campylobacter*-positive flock is slaughtered, it is likely that a large number of carcasses will become contaminated. According to a 2008 survey, most slaughterhouses in the EU are highly likely to have *Campylobacter*-positive broilers as starting material.[5]

A number of measures can be taken to protect consumers from campylobacteriosis and include scheduled slaughter, logistical slaughter or after-slaughter treatment such as disinfection with chlorinated water, which is used in the U.S., or steam treatment. Disinfection using chlorine is not perceived as a positive approach, with consumer sentiment in the EU opposing imports of "chlorine chicken."[6]

Scheduled slaughter requires identification of flocks positive for *Campylobacter* and subjecting carcasses from these flocks to *Campylobacter*-reducing measures. Flocks must be sampled prior to slaughter, with results from the testing available before transport to the slaughterhouse. For scheduled slaughter, the samples should be taken and test results delivered as close to slaughter as possible.

With logistical slaughter, flocks are slaughtered in order of contamination severity with negative flocks prioritized after positive flocks; the intention is to prevent cross-contamination of carcasses. While this approach has been widely adopted to better control *Salmonella*, a study of the process has indicated that surprising levels of contamination still occur.[7]

The time between testing flocks at the farm and delivery to the slaughterhouse may be two weeks or more. Even if testing is performed within days of slaughter, flocks may become positive during the interim.

Rapid Identification with Immunoassay Testing

Since *Campylobacter* can spread to an entire flock in a matter of days, testing with a rapid turnaround time is essential. Unfortunately, current *Campylobacter* detection methods have significant shortcomings. The most commonly used techniques to test food products for *Campylobacter* are traditional methods based on culture media.

The majority of *Campylobacter* spp. have low biochemical activity; therefore, identification is difficult on phenotypic characteristics. The transport of samples to the lab can increase the risk of false negatives if transportation conditions are inadequate for *Campylobacter* survival. The standard detection method is enrichment for 48 hours in a microaerophilic environment, followed by isolation on selective agars for 48 hours in a microaerophilic environment. Results are, therefore, only available after four to five days. Both culture steps have to be carried out in a microaerophilic environment.

These methods are time-consuming as well as labor-intensive. Optimal growth conditions are a key requirement for rapid screening and identification. To cultivate microaerophiles in the food testing labs, Anaerocult® (see **Figure 2**) products comprise systems for producing the anaerobic, microaerobic and capneic growth conditions that are required for anaerobiosis. The components of the Anaerocult reagent mixture react with water to chemically bind all or part of the atmospheric oxygen in a given volume of air (anaerobic jar or special incubation bag), simultaneously releasing carbon dioxide. The resulting growth environment is rich in carbon dioxide and either devoid of oxygen (anaerobic) or oxygen deficient (microaerobic).

Figure 2: Anaerocult® products for creating anaerobic, microaerobic and capneic growth conditions

Rapid detection methods are enabling the poultry industry to shift from contamination response to earlier detection and deliver on the promise of "farm to fork" protection as envisioned by regulatory authorities to help protect product integrity and reduce risk to consumers. While real-time PCR detection is fast, it requires capital-intensive instrumentation (e.g., a thermocycler) and trained personnel.

Simple-to-use Singlepath® lateral flow tests for *Campylobacter* detection (see **Figure 3**) deliver results in just a few minutes and are used following 48 hour enrichment. They allow the poultry industry to identify the presence or absence of contamination much faster than traditional culture methods, enabling a more cost-efficient and timely response. The tests are AOAC Research Institute–approved and optimized for use in a laboratory facility for end-product release testing.

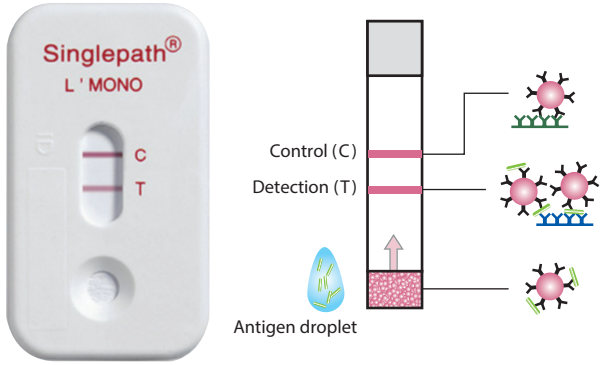
Figure 3: Singlepath® lateral flow tests, a rapid immunological system

Singlepath *Campylobactor* test result negative

Singlepath *Campylobactor* test result positive

The tests are based on immunochromatographic principles and use antibody-linked colloidal gold particles to react specifically with its complementary antigenic determinant to provide a visual reaction read-out. Results are delivered without the need for expensive instrumentation or trained staff. Results are clearly displayed in a yes/no format within minutes after sample application (see **Figure 4**).

Figure 4: Singlepath® *Campylobacter* lateral flow tests provide a rapid yes/no answer



References

1. World Health Organization (2012). Initiative to Estimate the Global Burden of Foodborne Diseases. http://www.who.int/foodsafety/foodborne_disease/ferg/en/index.html
2. European Food Safety Authority (2014). Campylobacter. http://www.efsa.europa.eu/en/topics/topic/campylobacter.htm
3. Centers for Disease Control. http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/
4. Incidence of Campylobacteriosis Stabilizes in Europe. Global Meat News. January 30, 2015. http://www.globalmeatnews.com/Safety-Legislation/Incidence-of-campylobacteriosis-stabilises-in-Europe
5. Campylobacter Control During Poultry Slaughter and Processing. 2011-05-08 http://www.wattagnet.com/Campylobacter_control_during_poultry_slaughter_and_processing.html
6. Merkel Says Will Prevent Chlorine-washed Chicken Imports from U.S. Reuters. May 24, 2014 http://www.reuters.com/article/2014/05/24/us-usa-eu-trade-merkel-idUSBREA4N07X20140524
7. de Zutter, et al., Logistical Slaughter Still Allows Carcass Contamination. World Poultry - Salmonella & Campylobacter Special 2005
8. http://www.who.int/mediacentre/factsheets/fs139/en/

Order Information

Description	Cat. No.
Singlepath *Campylobacter*	1.04143.0001
Anaerocult® C	1.16275.0001

Serratia

By Santiago Redondo-Salvo[1], María Lázaro-Díez[2], Adrián Fernández-Sánchez[2], Jose Ramos-Vivas[2]
[1] Aragón Institute of Engineering Research (I3A), University of Zaragoza, Spain
[2] IDIVAL Research Institute, Marqués de Valdecilla Universitary Hospital, Santander, *Spain*.
jvivas@idival.org

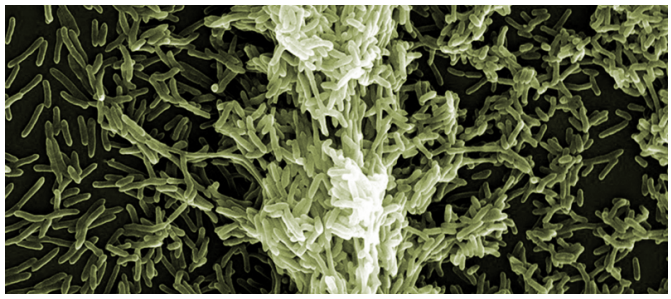
Dangerous Bacteria of the Genus *Serratia*

Serratia species are Gram-negative bacteria that belong to the Enterobacteriaceae family and are capable of thriving in a diverse number of environments that include soil and water as well as those associated with animals, plants, insects and humans.[1] Currently accepted species and subspecies in the genus *Serratia* include *S. entomophila*, *S. ficaria*, *S. fonticola*, *S. glossinae*, *S. grimesii*, *S. liquefaciens*, *S. marcescens* subsp. *marcescens*, *S. marcescens* subsp. *sakuensis*, *S. nematodiphila*, *S. odorifera*, *S. plymuthica*, *S. proteamaculans*, *S. quinivorans*, *S. rubidaea* and *S. ureilytica*. The genus contains plant pathogens, insect and nematode pathogens, coral pathogens, plant growth-promoting rhizobacterium, and insect symbionts.[2] *S. marcescens*, the species most likely to be recovered from human infections, contains several *S. marcescens* biogroups and biovars; their differential characteristics are summarized in the current edition of Bergey's Manual of Systematic Bacteriology. *S. liquefaciens* is part of the *S. liquefaciens* complex, along with *S. grimesii*, *S. proteamaculans*, and *S. quinivorans*. The precise identification of the members of the complex is very difficult for routine microbiology laboratories to distinguish accurately, and several reports should be assumed to include the other members of the complex unless this possibility has been specifically excluded by the use of molecular taxonomic methods, or, more recently, by proteomic approaches. For example, species identification by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry is able to differentiate between the three species of the *S. liquefaciens* complex. Some MALDI-TOF systems use *Serratia liquefaciens* (ATCC® 27592™), *Serratia grimesii* (ATCC® 14460™), *Serratia proteamaculans* subsp. *proteamaculans* (ATCC®19323™) and *Serratia proteamaculans* subsp. *quinovora* (ATCC® 33765™) as reference strains.[3] The ubiquity of *Serratia* spp is largely attributed to a variety of compounds that they release as extracellular products into the surrounding microenvironment. Moreover, adhesion and biofilm formation are also characteristic of many *Serratia* species. Growth in biofilms enables bacterial populations to survive better in hostile environments and during host infections (i.e., in the presence of antibiotics), increasing the probability of causing infections (**Figure 1**).

Human Pathogens

Some *Serratia* species are opportunistic bacteria for humans, with *Serratia marcescens* and *S. liquefaciens* being frequently encountered in nosocomial infections.[4,5] Moreover, *Serratia* species are inherently resistant to several antibiotics and are capable of readily acquiring antimicrobial resistance.[6] *Serratia* marcescens is part of normal colon flora. This organism also typically colonizes the respiratory and urinary tracts and causes infections in those organ systems. *S. liquefaciens* is an increasingly recognized cause of transfusion-related sepsis, and has been reported as a cause of meningitis, thrombophlebitis, corneal ulcers and other infections.

Figure 1: Representative scanning electron micrograph of a bacterial biofilm formed by *S. liquefaciens* on plastic surfaces at the liquid–air interface in LB medium



Virulence and Genomic Analysis

All of the species in the genus are motile at certain temperatures, toxins (**Figure 2**) and quorum sensing (QS) have been described for some species.[3, 7, 8] *S. marcescens* and *S. liquefaciens* are capable of producing well-known virulence factors such as pili (or fimbriae) for adherence (**Figure 3**).[3,9]Moreover, many of the clinical isolates of *S. marcescens* and *S. liquefaciens* carry chromosomal and plasmid-encoded genetic determinants specifying resistance to a wide range of antibiotics including extended-spectrum ß-lactamases (ESBLs) or metallo ß-lactamases (MBLs), but their genomic plasticity is yet to be fully understood.[10, 11, 12]

Figure 2: Example of a scanning electron micrograph of the cell death of an epithelial cell (green) infected with *S. liquefaciens* (red). The plasma membrane of the infected cell has lost its integrity

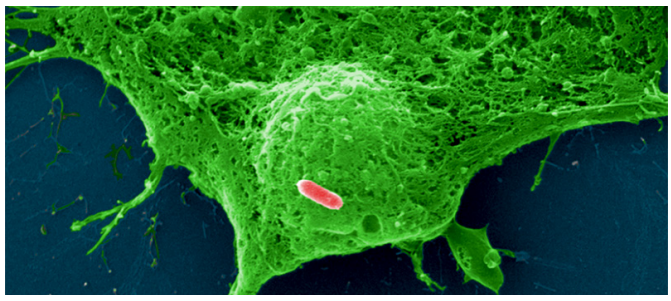
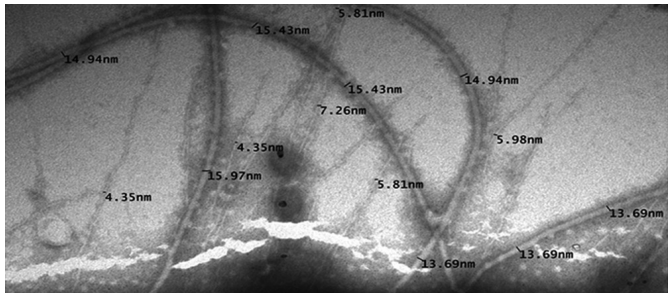


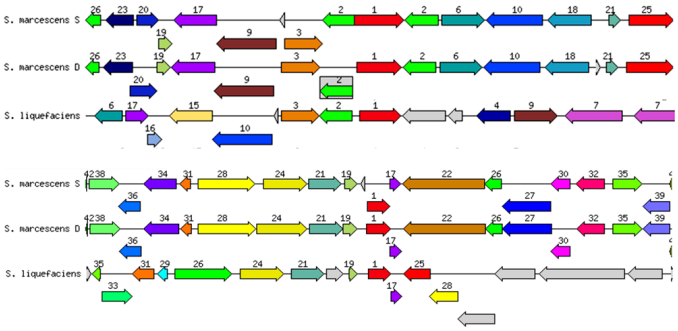
Figure 3: Example of a transmission electron micrograph of negatively stained bacteria grown in LB medium

Note the attachment of pili (low diameters) at the bacterial surface together with flagella (high diameters).



Recently, we have introduced the complete genome sequence of a clinical *S. liquefaciens* (GenBank accession number NZ_CP011303)[12] (PacBio single-molecule real-time (SMRT) sequencing). The final complete genome resulted in a single contig of 5,326,657 bp, with a total GC content of 55.2%. A total of 5,844 protein-coding sequences were predicted. For the interspecies comparison of selected proteins of interest, the following three complete genomes were used: *S. marcescens* strain SM39, isolated from a septicemic patient[13] (GenBank accession number NZ_AP013063), *S. marcescens* strain DB11, a spontaneous streptomycin-resistant derivative of strain Db10, which was isolated from a moribund Drosophila melanogaster in Sweden (GenBank accession number NZ_HG326223) and a human isolate HUMV-21 (GenBank accession number CP011303.1). Genome-wide comparative and interstrain clustering analyses of protein coding sequences (CDSs) were performed, yielding interesting information. Some examples of a conserved β-lactamase and the plasmid partitioning protein ParA are show in **Figure 4**. These data will accelerate research on *S. liquefaciens* in numerous domains and provide new insights into the genetic mechanisms responsible for the emergence of pathogens highly resistant to multiple antimicrobial agents.

Figure 4: Comparison of the SM39 and Db11 and HUMV-21 genomic loci bearing (in red, n °1) a conserved β-lactamase (up) and the plasmid partitioning protein ParA (down)



References

1. Mahlen, S. D. Serratia Infections: From Military Experiments to Current Practice. *Clinical Microbiology reviews* **2011**, *24* (4):755–91.
2. Petersen, L. M.; Tisa, L. S. Friend or Foe? A Review of the Mechanisms That Drive *Serratia* Towards Diverse Lifestyles. *Canadian Journal of Microbiology* **2013**, *59* (9):627–40.
3. Remuzgo-Martinez, S.; Lazaro-Diez, M., Mayer, C.; Aranzamendi-Zaldumbide, M.; Padilla, D.; Calvo, J., et al. Biofilm Formation and Quorum-Sensing-Molecule Production by Clinical Isolates of *Serratia liquefaciens*. *Applied and Environmental Microbiology* **2015**, *81*(10):3306–15.
4. Roth, V. R.; Arduino, M. J.; Nobiletti, J.; Holt, S.C.; Carson, L. A.; Wolf, C. F., et al. Transfusion-related Sepsis Due to *Serratia liquefaciens* in the United States. *Transfusion* **2000**, *40*(8):931–5.
5. Dessi, A.; Puddu, M.; Testa, M.; Marcialis, M. A.; Pintus, M. C.; Fanos, V. *Serratia marcescens* Infections and Outbreaks in Neonatal Intensive Care Units. *Journal of Chemotherapy* **2009**, *21*(5):493–9.
6. Traub, W. H. Antibiotic Susceptibility of *Serratia marcescens* and *Serratia liquefaciens*. *Chemotherapy* **2000**, *46*(5):315–21.
7. Van Houdt, R.; Givskov, M.; Michiels, C.W. Quorum Sensing in *Serratia*. *FEMS Microbiology reviews* **2007**, *31*(4):407-24.
8. Remuzgo-Martinez, S.; Aranzamendi-Zaldunbide, M.; Pilares-Ortega, L.; Icardo, J.M.; Acosta, F.; Martinez-Martinez, L., et al. Interaction of Macrophages with a Cytotoxic *Serratia liquefaciens* Human Isolate. *Microbes and Infection* **2013**, *15*(6-7):480-90.
9. Singh, B.R.; Singh, Y.; Tiwari, A.K. Characterisation of Virulence Factors of *Serratia* Strains Isolated from Foods. *International Journal of Food Microbiology* **1997**, *34*(3):259-66.
10. Boyd, D.; Taylor, G.; Fuller, J.; Bryce, E.; Embree, J.; Gravel, D., et al. Complete Sequence of Four Multidrug-Resistant MOBQ1 Plasmids Harboring blaGES-5 Isolated from *Escherichia coli* and *Serratia marcescens* Persisting in a Hospital in Canada. *Microbial Drug Resistance* **2015**, *21*(3):253-60.
11. Iguchi, A.; Nagaya, Y.; Pradel, E.; Ooka, T.; Ogura, Y.; Katsura, K., et al. Genome Evolution and Plasticity of *Serratia marcescens*, an Important Multidrug-resistant Nosocomial Pathogen. *Genome Biology and Evolution* **2014**, *6*(8):2096-110.
12. Lazaro-Diez, M.; Acosta, F.; Remuzgo-Martinez, S.; Ocampo-Sosa, A.; Ocejo-Vinyals, J.G.; Bravo, J., et al. Whole-Genome Sequence of *Serratia liquefaciens* HUMV-21, a Cytotoxic, Quorum-Sensing, and Biofilm-Producing Clinical Isolate. *Genome announcements* **2015**, *3*(3).
13. Nakamura, T.; Shibata, N.; Doi, Y.; Okuda, K.; Nakata, C.; Heijyo, H., et al. IMP-1 Type metalo-beta-lactamase Producing *Serratia marcescens* Strains Isolated from Blood Culture between 1991 to 2000. *Kansenshogaku zasshi* **2002**, *76* (4):246-53.
14. Flyg, C.; Kenne, K.; Boman, H.G. Insect Pathogenic Properties of *Serratia marcescens:* Phage-resistant Mutants with a Decreased Resistance to Cecropia Immunity and a Decreased Virulence to Drosophila. *Journal of General Microbiology* **1980**, *120*(1):173-81.

Table: Characteristic properties and test for *Serratia marcescens*

Typical Properties	Test	Cat. No.
Hydrolyzation of gelatin	Nutrient Gelatin	70151
DNase positive	DNase Test Agar with Toluidine Blue	D2560
	Deoxyribonuclease Test Agar	70136 or 30787
Chitinase positive	4-Nitrophenyl N-acetyl-β-D-glucosaminide substrate	N9376
Lipase positive	5-Bromo-4-chloro-3-indolyl nonanoate substrate	53657
Nitrate reduction	Nitrate Reduction Test	73426
Gram negative	Gram Staining	77730
Lactose negative	MacConkey Broth Purple	16377
	MacConkey Agar No. 1	70143
	Lactose Disks	28816
	ONPG Test	49940
Glucose positive	Dextrose Disks	63367
Indole negative	Kovac's Reagent	67309 or 60983
Urease negative	Urea Agar Base (Christensen)	U1757
Motile	Microscopy	
No hydrogen sulfide production	Hydrogen Sulfide Test Strips	06728
Lysine decarboxylase	Decarboxylase Broth Base, Moeller	D2935
Ornithine decarboxylase	Decarboxylase Broth Base, Moeller	D2935
Oxidase negative	Oxidase Strips	40560
	Oxidase Test	70439
Catalase positive	Catalase Test	88597

Order/Customer Service: sigma-aldrich.com/order
Technical Service: sigma-aldrich.com/techservice
Development/Custom Manufacturing Inquiries SAFC® safcglobal@sial.com
Safety-related Information: sigma-aldrich.com/safetycenter

3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

RZW
84417
1036