



Microbiology Focus

Volume 3.3, 2011



Bacteremia



Aggressive infection on red blood cell in humans

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Microorganisms are Photogenic

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

As 2010 came to a close and the new year began, many Microbiologists sent photos of their beloved microbes.

The aim of the photo competition was to encourage microbiologists to promote their work, with the condition that entries should illustrate any microorganisms (living or dead) or a microbiologist in action at work. We received numerous entries and the decision was not an easy one for the jury. Sigma-Aldrich thanks all of the microbiologists who sent us their impressive images, along with their interesting and amusing descriptions. Sigma-Aldrich would also like to thank our independent jury members Dr. Antje Breitenstein (BioSolutions Halle GmbH) and Prof. Dr. Mohammad Manafi (Medical University of Vienna). The five winners received prizes such as an iPod Nano, a Swiss army knife, a USB jump drive, and a laser pointer. I hope our winners enjoy their prizes as much as we have enjoyed hosting the photo competition!

1st Place

Aggressive infection on red blood cell in humans (Cover image).

Arsalan Daudi

Royal Holloway University of London

2nd Place

*Microcolony with a toroidal structure of the pathogen, *Aeromonas hydrophila*, on the wall of a well, in a 96-well plate.*

Jose Ramos Vivas

Instituto de Formación e Investigación Marqués de Valdecilla IFIMAV

3rd Place

**Chromatium okenii* with intracellular sulfur droplets. Cell length is approximately 10 micrometers.*

Helmut Brandl

University of Zurich

4th Place

**Rhodococcus equi* (intracellular bacterium in red-orange) infection in human epithelial cells undergoing mitosis. DNA stained with DAPI and cellular actin in green.*

Jose Ramos Vivas

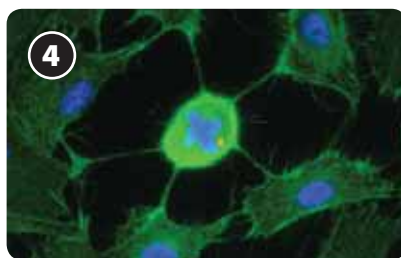
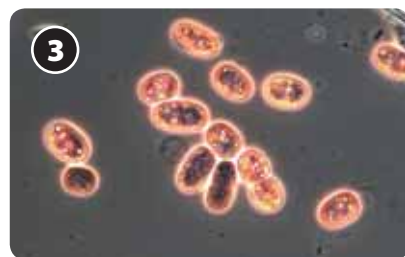
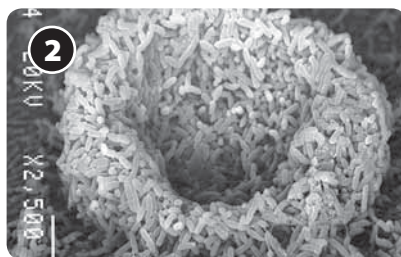
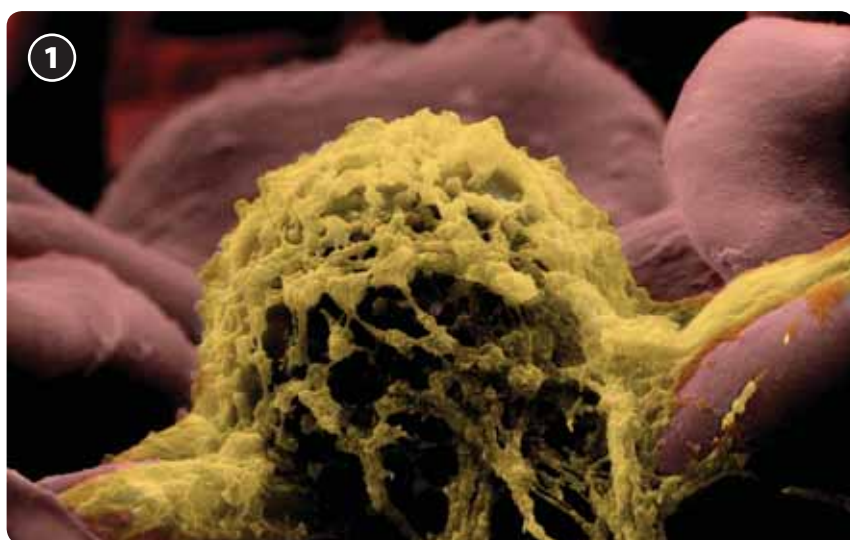
Instituto de Formación e Investigación Marqués de Valdecilla IFIMAV

5th Place

**Fusarium golden eye*.*

Ela Suchowilska

University of Warmia and Mazury in Olsztyn-Poland



More details and the ranking of the best 15 images can be found on our website: sigma-aldrich.com/mibi-competition

Bacteremia

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

Bacteria in the blood pose a severe health problem.

The blood of a healthy person is sterile; however, if some viable bacteria enters into the bloodstream, it disseminates quickly causing serious infection such as endocarditis, osteomyelitis, pneumonia or meningitis. Even a strong immune response may not be able to prevent sepsis, which can proceed to septic shock and often results in a high mortality rate. Sepsis is a blood poisoning caused by bacteria, fungi, their toxins, or viruses, resulting in a complete inflammatory response; severe sepsis includes the presence of organ dysfunction. In such a case, immediate treatment with the most effective antibiotic is critical.

To detect such bacteria, a blood culture must be taken. Even the strains of bacteria that are normally non-threatening, such as *E.coli*, can exacerbate the infectious condition arising from the sepsis. In some cases, pathogens are introduced at a minor site of infection but then pass through membranes and invade into the blood. In many other cases, the infection is caused by exposure to contamination in a hospital settings, such as through the insertion of catheters or during surgical procedures.

Blood cultures

A minimum of 10 mL of blood is taken and 3-10 mL are transferred into blood culture bottles containing a medium such as Brain Heart Infusion Broth. One of these bottles contains a medium for anaerobes with carbon dioxide, while the other bottle contains a medium for aerobes and oxygen. Multiple blood samples are taken in intervals of at least 30 minutes. The culture bottles are incubated at 37 °C for several days and monitored for changes in turbidity or in gas mixture composition. The bacterial identification is initiated as soon as a change is detected. Even today, one of the first steps continues to be the performance of a gram staining (see also Gram Staining kit Fluka 77730); the blood culture is subcultured on agar plates for further differentiation, identification and antibiotic sensitivity testing. This testing is then applied towards developing an efficient treatment plan using the most effective antibiotic.

Table 2: Some typical non-selective media for general bacteria cultivation

Cat. No.	Media	Cat. No.	Media
A3340	AC Agar	01477	Nutrient Agar No 2*
93207	AC Broth	70116	Nutrient Agar No 2*
70133	Blood Agar (Base)	04163	Nutrient Agar No 2, Vegitone
B1676	Blood Agar Base No. 2	17179	Nutrient Agar pH 6.0 with 0.8% NaCl
70138	Brain Heart Infusion Agar	N9405	Nutrient Agar pH 6.8
53286	Brain Heart Infusion Broth	70122	Nutrient Broth No 1
22089	Casein peptone Lecithin Polysorbate Broth	70123	Nutrient Broth No 2*
22095	CASO Agar	70149	Nutrient Broth No 3
22098	CASO Broth	16336	Nutrient Broth No 3, Vegitone
27688	Columbia Agar	78104	Nutrient Broth No 5
05121	Heart Infusion Broth	03856	Nutrient Broth No. 4*
19344	LB Agar, Vegitone	17181	Nutrient Broth pH 6.9 without NaCl
28713	LB Broth, Vegitone	77185	Peptone Water
52062	LB Agar, high salt	70179	Peptone Water
51208	LB-Broth, high salt	77187	Peptone Water, phosphate-buffered
07233	Maximum Recovery Diluent	40893	Peptone Water, phosphate-buffered, Vegitone
70191	Mueller Hinton Agar	70152	Plate Count Agar
70192	Mueller Hinton Broth	88588	Plate Count Agar according to Buchbinder et al.
90922	Mueller Hinton Broth 2 (Cation-Adjusted)		
97580	Mueller-Hinton Agar 2		
70148	Nutrient Agar		

Figure 1: Blood cultures



Table 1: Some typical media used for anaerobic bacteria

Cat. No.	Media
15997	Brewer Agar*
B2551	Brewer thioglycollate medium
B2926	Brucella Agar with Hemin and Vitamin K
91903	Bryant and Burkey Medium
27546	Clostridial Nutrient Medium*
60865	Cooked Meat Broth
61724	Liver Broth*
46379	Meat Liver Agar
91019	Schaedler Agar
70157	Thioglycollate Broth (USP Alternative)
28976	Thioglycollate Medium with K Agar
T3938	Tryptone Soya Broth without Dextrose
W1761	Wilkins Chalgren Anaerobic Agar

*not available in USA.

Cat. No.	Media
19718	Plate Count Agar, Vegitone
17209	R-2A Agar
S4681	Standard Nutrient Broth No. 1
90404	Thioglycollate Broth with Resazurine
22091	Tryptic Soy Agar
14432	Tryptic Soy Agar, Vegitone
22092	Tryptic Soy Broth
51228	Tryptic Soy Broth No. 2
41298	Tryptic Soy Broth, Vegitone
51414	Tryptic Soya Agar with Polysorbate 80 and Lecithin
93655	Tryptone Agar*
70159	Tryptone Glucose Extract Agar
T2188	Tryptone Glucose Yeast Extract Agar
93657	Tryptone Medium
70194	Tryptone water
39964	Tryptone water, Vegitone
17221	Tryptone Yeast extract Agar
41960	Vegitone Infusion Broth

*not available in USA.



A typical procedure to identify Gram-negative bacilli

A positive blood culture is subcultured directly onto trypticase soy agar with 5 % sheep blood and onto MacConkey agar. A 0.5 mL sample of the broth is diluted in 2.5 mL sterile 0.85 % saline and then used to complete biochemical tests. Typically used biochemical tests are performed on Kligler iron agar (Fluka 60787), Simmons' citrate agar (Fluka 85463), Christensen's urea

agar (Fluka 27048), a phenylalanine agar (deaminase reaction, Fluka 78052), tryptone water (indole reaction, Fluka 70194), malonate broth (Fluka M8802) and oxidative–fermentative agar (Fluka 75315). All media are incubated overnight at 35 °C and the results are then interpreted the following day.

Did you know...

Staphylococcus aureus is very specific to human blood.

S. aureus can hide in human cells in a dormant stage (in which only minimal essential metabolism remains active) for at least two weeks without being detected by the immune system and where it is shielded from antibiotics. In addition, *S. aureus* has a high specificity to human blood, as it shows an enhanced ability to bind hemoglobin derived from humans as compared to other mammals. The destruction of hemoglobin is essential for *S. aureus* to obtain iron for its metabolism.



Figure 2: *S. aureus* on agar plate

Aeromonas hydrophila Microcolonies and Biofilms

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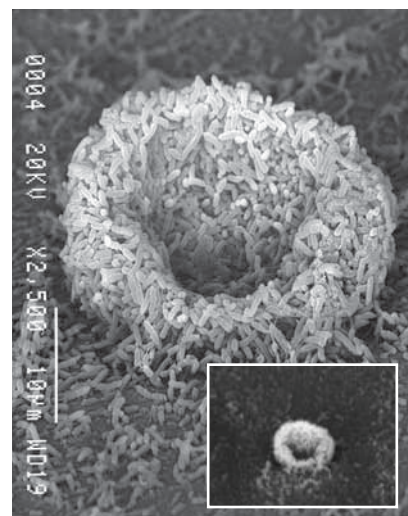
Organisation and biofilm formation of a potential pathogen.

Aeromonas hydrophila is a Gram-negative rod, motile, non-sporing and facultative anaerobic bacterium widely distributed in nature. It is an opportunistic pathogen of poikilothermic and homeothermic animals, including humans. As with other motile aeromonads, *A. hydrophila* may adhere to solid surfaces and form biofilms in aquatic environments. Typically, a biofilm is composed of microbial cells, extracellular polymeric substances (EPS) secreted by the cells, metabolic products, and a variety of colloidal and dissolved substances [1]. Bacteria growing in biofilms show specific structural and physiological properties and, remarkably, increased resistance to antimicrobial drugs [2-3]. All these features have been related to a higher persistence and severity of several bacterial diseases affecting human [4] and animal health [5], including that of aquacultured fish [6]. The particular features of the biofilms formed by virulent bacterial strains may facilitate a higher waterborne dispersal capacity, a feature that has been related to a higher risk of bacterial transmission and infectivity [7-8].

In this work, the morphology of microcolonies and biofilms formed by different *A. hydrophila* strains on the walls of U-bottomed polystyrene microtiter plates was studied by Scanning Electron Microscopy (SEM). To accomplish this, bacterial strains were grown on polystyrene microtiter plates in different culture media, for 12-24h, without shaking. After removing the culture media and washing with sterile water, bacteria were fixed at 4 °C for 1h directly inside the microtiter plates by adding 250 µl/well of cold 2% (vol/vol) glutaraldehyde in PBS. After fixation, the samples were dehydrated in graded ethanol series, freeze-dried using a critical point drier, and coated with gold in a sputter coater (Balzers). The samples were examined and photographed in a JEOL JSM 6100 scanning electron microscope working at 20 kV.

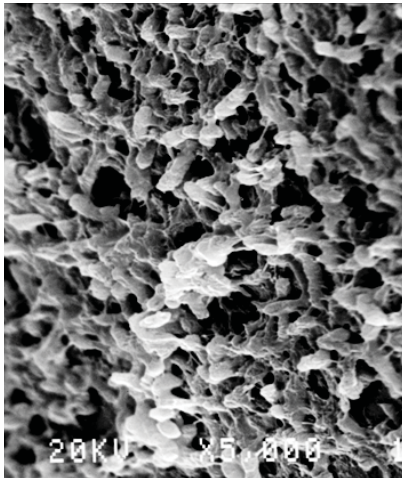
After 12 h of incubation, the three *A. hydrophila* strains were able to form microcolonies and early biofilms on the plastic surface. One strain formed microcolonies showing distinctive ring or “doughnut-like” morphologies, with protruding borders and a central hole (Figure 1), with scarce EPS production. Morphologically, the process of biofilm

Figure 1: *Aeromonas hydrophila* microcolony with a toroidal structure.



(Inset photo): A bacterial microcolony formed by a strain of *A. hydrophila* on the wall of plastic wells in a microtiter plate. On the lower right corner, the structure is shown at lower magnification.

Figure 2: Mature biofilm formed by *A. hydrophila*. Scanning electron micrographs of mature biofilms formed by an *A. hydrophila* strain on the wall of polystyrene wells after 24 h of incubation. Note the numerous openings on the even surface of the biofilm formed by bacteria.



formation described for the *A. hydrophila* strains discussed in this article resembled those described in the literature [9], including bacterial attachment to the substrate, formation of microcolonies (Figure 1), and maturation of the biofilm (Figure 2).

Doughnut, or ring-shaped, structures, such as those formed by the *A. hydrophila* strains have also been described for other bacterial species; this particular morphology is related to bacterial detachment from the centre of the microcolonies [8,10], an occurrence that might be facilitated by the scarcity in EPS production and the hydrophobic surface of some *A. hydrophila* virulent strains. Our studies indicate that *A. hydrophila* strains differ in virulence and cell surface phenotype, and they form microcolonies and biofilms with different morphological features. In some strains, these features may facilitate the microbe's waterborne dispersal and, therefore, its infectivity.

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Figure 3: Koi swimming in a water garden

Did you know...

Aeromonas is also a health threat to fish?

In aquariums and ponds, *aeromonas* growth is inhibited by beneficial nitrifying bacteria, with which they compete for the same nutritional source.



Aeromonas



Aeromonas Detection

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

Detection, identification, differentiation and cultivation of *Aeromonas*.

Aeromonas resembles Enterobacteriaceae both morphologically and biochemically. *Aeromonas* is a gram-negative, facultative anaerobic rod-shaped bacterium. The various species of *Aeromonas* can be divided into two groups: the mesophile and the psychrophile types. Most mesophiles, such as *A. hydrophila*, have polar flagella and are motile, while the psychrophiles are primarily non-motile because they do not possess flagella. The typical biochemical reactions are oxidase and catalase positive. *A. hydrophila* digests materials such as gelatin and haemoglobin and is resistant to ampicillin, chlorine, and cold temperatures. To isolate *Aeromonas* spp., blood agar supplemented with ampicillin and CIN (cefsulodin-irgasan-novobiocin) agar may be used. After incubation at 37 °C for 24–48 hours, an oxidase test is performed. Biochemical criteria are used to identify and differentiate *Aeromonas*.

Aeromonas may be present in a variety of aquatic environments, in both freshwater and brackish water. The organisms are known to be a possible cause of wound infections as well as gastroenteritis. They may be introduced to humans through ingestion of contaminated food products (mainly seafood and meat) or through open-wound exposure to contaminated water.

The most important species of this genus are *A. hydrophila*, *A. caviae*, and *A. veronii* biovar *sobria*, all of which are seen as opportunistic pathogens. They possess virulence factors such as endotoxins, enterotoxins, hemolysins and adherence factors and are known to be capable of building biofilms; however, the details about the pathogenic mechanisms are unknown. It is also assumed that only some strains are pathogenic.

Table 1: Biochemical Characteristics of *Aeromonas*

Test	<i>A. caviae</i>	<i>A. hydrophila</i>	<i>A. sobria</i>	<i>A. veronii</i>
Motility	+	+	+	+
Indole production	+	+	+	+
Esculin hydrolysis	+ (88%)	+	+ (17%)	+
Arabinose	+ (94%)	+ (97%)	-	-
Glucose with gas formation	-	+ (84%)	+ (83%)	+
Salicin	+	+ (91%)	-	+
Sucrose	+	+	+	+
Mannitol	+	+ (94%)	+	+
Inositol	-	-	-	-
Acetoin production	-	+ (94%)	+ (67%)	+
H ₂ S production	-	+	+	+
Histidine & Arginine utilization	+ (82%)	+	+ (17%)	+

Figure 1: Oxidase Strips



Table 2: Media used for isolation and differentiation of *Aeromonas*

Cat. No.	Name
17118	Aeromonas Isolation Agar (Base)
70133	Blood Agar (Base)
95760	CIN Agar (Cefsulodin-Irgasan-Novobiocin)
31433	DEV Gelatin Agar*
50875	GSP Agar
70151	Nutrient Gelatin
75315	OF Test Nutrient Agar*

* not available in the USA.

Table 3: Biochemical tests

Test	Cat. No.
Arabinose disks	80372
Barritt's Reagent A	29333
Barritt's Reagent B	39442
Bile Esculin Disks	80507
Catalase Test	88597
Dextrose disks	63367
Hydrogen Sulfide Test Strips	06728
Inositol	89614
Kovac's Reagent for indoles	67309
Kovac's Reagent for indoles	60983
Mannitol disks	94438
Methyl Red Voges Proskauer Broth	39484
O'Meara's reagent	07689
Oxidase Reagent acc.	07345
Gaby-Hadley A	
Oxidase Reagent acc.	07817
Gaby-Hadley B	
Oxidase Reagent acc.	18502
Gordon-McLeod	
Oxidase Strips	40560
Oxidase Test	70439
Salicin disks	92971
Sucrose disks	94309

Enterohemorrhagic *E. Coli* (EHEC)

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In May 2011, Germany experienced an increased incidence of haemolytic-uremic syndrome (HUS) caused by the often life-threatening EHEC.

Normally, *E. coli* is a harmless intestinal bacterium. However, some strains are able to produce a Shiga-like toxin, also known as verotoxin, which is able to cleave the n-glycosidic link to adenine in the 28S-rRNA. This iron-regulated reaction leads to cell death, bloody diarrhea, and possible kidney damage [1]. In addition, special core proteins (adhesins) have been found which give the EHEC the ability to adhere to intestinal epithelial cells [3]. In some cases, a heat stable toxin (astA gene) has been found on the bacteria chromosomes [5]. Hemolysin, catalase-peroxidase and the serine protease are additional virulence factors present in EHECs. The genes for these factors have been found on the plasmid pO157, where additional genes for the pathogenic mechanism are likely to be present as well [4].

The pathogenic genes accrue from an infection of *E. coli* from a bacteriophage or from contact with other verotoxin-producing bacteria [2]. The nomenclature of the different EHEC strains is based on the serological differentiation. The "O" from the O157:H7 refers to an antigen present on the cell wall, while the "H" pertains to the antigen found on the flagella.

EHEC is found in nature in animals, particularly in cows, which then serve as a source of contamination for raw meat and milk, as well as for vegetables (fertilization). Ingestion of undercooked meat is the primary route for infection; however, contact infection from animal to human or human to human is also possible, since the minimum infection dose is 10 – 100 cells.

Although *E. coli* O157:H7 is the most well-known pathogen causing bloody diarrhea, there are also other noteworthy strains of EHECs. *E. coli* O157:H7 is quite different from other pathogenic and non-pathogenic *E. coli*. It possesses a pilus protein (type of adhesin) for adhesion to the intestinal epithelial cells [6]. *E. coli* O157:H7 is not an enteroinvasive serotype [7] and demonstrates no or limited growth at 44-45.5 °C, particularly in the presence of 0.15% bile salts

Figure 1: HiCrome™ Enrichment Broth Base for EC O157:H7



[8]. It consistently produces EHEC hemolysin [9] and the heat stable toxin EAST1 [10]. There are multiple characteristics which help to differentiate it from other *E. coli* serotypes and from other types of bacteria. These features are also incorporated by diverse media to detect and differentiate *E. coli* O157:H7, as shown in Table 1.

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Table 1: List of Sigma-Aldrich media for detection of enteropathogenic *E. coli*

Name	Cat. No.
Non-selective + differential system	
Mucate Broth	17178
Selective + differential systems	
<i>E. coli</i> O157:H7 MUG Agar	44782
HiCrome™ EC O157 Agar* - works also for O104 serotypes	39894
Optional supplement: 0.25 mL/L 1% potassium tellurite solution (Cat. No. 17774)	
HiCrome EC O157:H7 Selective Agar, Base*	72557
works also for O104 serotypes	
Supplement: 1 vial/L of HiCrome ECO157:H7 Selective Supplement (Cat. No. 44931)	
HiCrome Enrichment Broth Base for EC O157:H7*	80330
works also for O104 serotypes	
HiCrome MacConkey-Sorbitol Agar*	83339
Supplement: 2 vial/L Tellurite-Cefixime Supplement (Cat. No. 77981)	
MacConkey-Sorbitol Agar	88902
Optional supplement: 2 vial/L CT-Supplement (Cat. No. 77981)	
Selective media	
mEC Broth with Novobiocin*	71882
Modified Tryptone Soya Broth	08069
Supplement: 1 vial/L VCC Selective Supplement (Cat. No. 80704)	
mTSB Broth with Novobiocin	76704

* not available in the USA.



EHEC

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