Microbiology Focus

Volume 4.4, 2012

Fluka Analytical

Analysis of Meat and Meat Products

Salmonella enteritidis with flagella on poultry meat. This zoonotic microorganism can cause salmonellosis (food poisoning) in humans.

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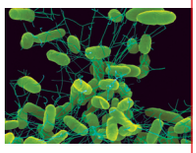


Analysis of Meat and Meat Products

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

Microbiological testing is important for determining the quality and safety of meat and meat products.

To control the quality, safety and hygiene of meat and meat products, it is recommended butchers, meat processing plants, and slaughterhouses follow some guidelines for self-regulation. The requirements vary based on the size of the company. Generally, the product group determines which microbes are of concern (**Table 1**) since both the meat type and the production process serve as the basis for possible contamination. It is known, for example, that raw sausage often contains *Listeria monocytogens* and chicken is a possible source of *Salmonella* and *Campylobacter* (**Figure 2**). For some meat products, pathogens or spoiling organisms are not the only issue. For example, the production of salami requires a large quantity of lactic acid bacteria, and later in the process, needs beneficial molds for the formation of a protective skin (white coat to avoid photo-oxidation and rancidity in the fat).

One important parameter is the number of **aerobic mesophiles**. This group contains all aerobic organisms with optimal growth at 35-40 °C. These microbes are not all pathogens, but they are indicative of the overall quality of the meat (**Figure 3**). This parameter is also called "total count" and is made by inoculation of classical media (plate count agar). The meat sample is added to a diluent (e.g. Maximum Recovery Diluent), as specified by ISO 6887-1, and then processed in a Stomacher. The sample should be diluted to a concentration of 15-300 colonies and then 1 mL of the diluted sample is put into a petri dish. 12 to 15 mL of the molten, sterile and cooled (44-47 °C) medium is added and then incubated aerobically at 30 °C for 72 hours. After incubation, the colonies on the plates are counted and the numbers are calculated and correlated to the amount of sample in colony forming units (cfu) per gram. More details and information can be found under ISO 4833:2003.

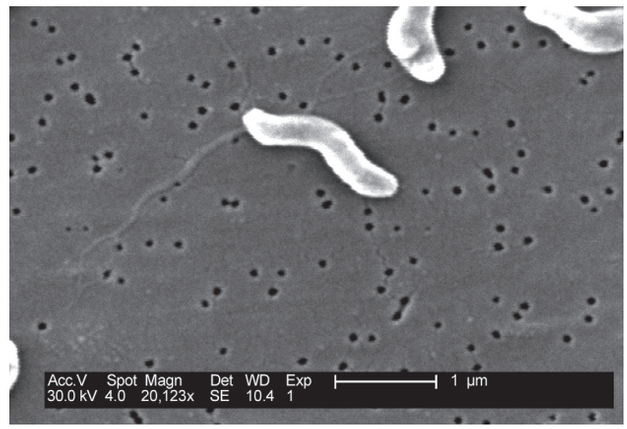


Figure 2: The Gram-negative *Campylobacter jejuni* is fragile. It cannot tolerate drying and can be killed by oxygen. It grows only if there is less than the atmospheric amount of oxygen present. Freezing reduces the number of *Campylobacter* bacteria present on raw meat. (Source: CDC; Dr. Patricia Fields, Dr. Collette Fitzgerald, Janice Carr 2004).

Another important organism is ***E. coli***, which is also an indicator for fecal contamination. Pathogen species, such as the EHECs, are a real danger to human health. Here the ISO 16649-2 recommends a general count of the *E. coli* with TBX Agar. This medium is made selective with the addition of bile salts and also contains a confirmation step with its chromogenic substrate to detect β-glucuronidase. The result is the colonies appear as typical blue colonies, which are quite easy to count. For further selectivity, the incubation temperature is elevated to 44 °C. If stressed cells are anticipated, it is recommended to begin with four hours at 37 °C and then increase the temperature to 44 °C. The total incubation time takes 18 to 24 hours.

The most important pathogen related to meat is ***Salmonella***. The ISO 6579:2002/A1:2007 proposes a nonselective enrichment with Buffered Peptone Water followed by selective enrichment on two different media (Rappaport Vassiliadis Broth and Muller-Kauffmann Tetrathionate Broth). These were developed because *Salmonella* is often accompanied by considerably larger numbers of other *Enterobacteriaceæ* or other species, and a nonselective enrichment is necessary to detect low numbers of *Salmonella* with respect to rescued injured cells. In a further step, a selective isolation is made by XLD agar and a complementary medium like *Salmonella* Chromogen Agar or XLT4 agar. For confirmation, the presumptive colonies are streaked out on Nutrient Agar No. 2, undergo biochemical testing on TSI, and finally serological tests are made.

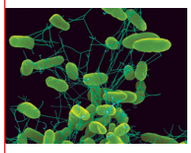
Did you know…

without lactic acid bacteria we would not have any salami?

Samelis et al. found a total of 348 lactic acid bacteria from five batches of naturally fermented dry salami. They analyzed flora at various stages of ripening.

Int J Food Microbiol., 179-96, 1994

Figure 1: Salami



Listeria monocytogenes is found on both raw meat and on cooked meat products. This bacterium is a real survivor and grows at low temperatures and other stringent conditions. The ISO 11290-1 provide, therefore, a general four-step process: enrichment, identification, isolation and confirmation. The samples are directly enriched in a selective broth (Fraser broth), and Oxford Agar and PALCAM Listeria Selective Agar are used as identification plate media. Afterwards the presumptive *Listeria monocytogens* colonies are taken and purified on Tryptone Soya Yeast Extract Agar plates and then confirmed by a biochemical test like Gram staining, Listeria Motility Medium, Carbohydrate Consumption Broth and Blood Agar Base No. 2.

Staphylococcus aureus is mainly a problem in raw meat, meat salads, and convenience foods which have undergone processing. A serious problem is that they have found multidrug-resistant strains on meat. ISO recommends, under the norms of 6888, the Baird Parker Agar for detection and enumeration of lipolytic and proteolytic activity and the ability to reduce tellurite to metallic tellurium (egg yolk tellurite supplement EN-ISO 6888-1: 1999). With the RPF Supplement, the coagulase activity and the ability to reduce tellurite is detected (EN-ISO 6888-2:2000). At the end, a resulting CFU per gram sample is calculated.

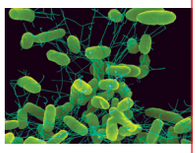
Product Group	Microbes
• Minced meat, mechanically recovered meat	• *Aerobic mesophilic organisms* • *Escherichia coli* • *Salmonella*
• Not / ready to eat meat	• *Escherichia coli* • *Salmonella*
• Raw, ready to eat meat products • Raw sausages, raw salting meat • Cooked meat products	• *Listeria monocytogenes*
• Cured and smoked sausages • Fermented raw sausages	• *Salmonella*
• Meat salad, convenience meat products	• *Aerobic mesophilic organisms* • *Escherichia coli* • *Coagulase positive Staphylococci*

Table 1: Meat products and the problematic microbes (source: SFF, Swiss Meat Professional Union)

Microbes	Cat. No.	Media	ISO Reference
Aerobic Mesophiles	70152	Plate Count Agar	4833:2003
	88588	Plate Count Agar according to Buchbinder et al.	4833:2003
	68414	Plate Count Agar according to Buchbinder et al.*	4833:2003
Campylobacter	B2426	Blood Free *Campylobacter* Selectivity Agar Base (Modified CCDA – Preston)	10272-1:2006
	67454	Bolton Broth Base	10272-1:2006
	92499	Triple Sugar Iron Agar (acc. to ISO)*	10272-1:2006
Cl. Perfringens/Clostridium sulfite reducers	93745	TSC Agar*	7937:2004
	93745	TSC Agar*	15213:2003
Coliforms	17349	Lauryl sulfate Broth	4831:2006
	17213	Violet Red Bile Glucose Agar without Lactose	4832:2006
E. coli	44653	EC Broth*	7251:2005
	72557	HiCrome™ EC O157:H7 Selective Agar, Base	16649-2:2001
	17349	Lauryl sulfate Broth	7251:2005
	88902	MacConkey-Sorbitol Agar	16654:2001
	17171	Mineral Modified Glutamate Broth (Base)	16649-3:2005
	76704	mTSB Broth with Novobiocincin	16654:2001
	70116	Nutrient Agar No. 2	16654:2001
	72557	TBX Agar	16649-2:2001
	09136	Tryptophan Broth	16654:2001
Enterobacteriaceaea	08105	Buffered Peptone Water (ISO)	21528-1:2004
	17213	Violet Red Bile Glucose Agar without Lactose	21528-1:2004
	17213	Violet Red Bile Glucose Agar without Lactose	21528-2:2004
Salmonella/Shigella	08105	Buffered Peptone Water (ISO)	6579:2002
	27048	Christensen's Urea Agar	6579:2002
	41932	L-Lysine decarboxylation Medium	6579:2002
	39484	Methyl Red Voges Proskauer Broth	6579:2002
	43052	Muller-Kauffmann Tetrathionate Broth, Base (ISO)	6579:2002
	89176	Muller-Kauffmann Tetrathionate Novobiocin Broth*	6579:2002
	70116	Nutrient Agar No. 2	6579:2002
	04584	Rappaport Vassiliadis Broth acc. to DIN EN ISO 6579:2002	6579:2002
	79890	Semi-solid Nutrient Agar ISO 6579:2002*	6579:2002
	92499	Triple Sugar Iron Agar (acc. to ISO)*	6579:2002
	92499	Triple Sugar Iron Agar (acc. to ISO)*	21567:2004
	09136	Tryptophan Broth	6579:2002
	14781	XLD Agar ISO 6579:2002	6579:2002
Staph. aureus	79893	Baird Parker Agar Base (RPF)	6888-2:1999
	79893	Baird Parker Agar Base (RPF)	6888-1:1999
	53286	Brain Heart Infusion Broth	6888-1:1999
	69527	Modified Giolitti and Cantoni Broth (ISO)*	6888-3:2003
Yersinia	22095	CASO Agar	10273:2003
	70116	Nutrient Agar No. 2	10273:2003
	22091	Tryptic Soy Agar	10273:2003

* Not sold in U.S.A.

Table 2: Some media for meat contamination control recommended by ISO. To find more media (e.g. chromogenic media) pertaining to bacteria control for meat, visit *sigma-aldrich.com/microbiology.*



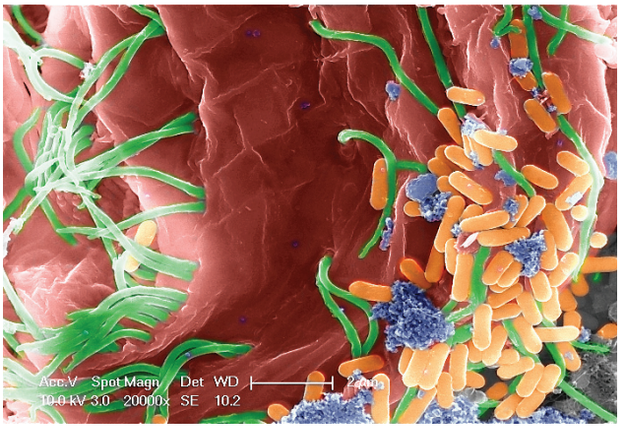
And The Winner Is…

In Microbiology Focus Volume 4.2 we invited you to take part in our Fluka Microbiology Photo Competition and got some nice images from all over the world. The winning pictures are shown with descriptions of what is being depicted. The complete list can be seen on our website *sigma-aldrich.com/microbiology*. Sigma-Aldrich would like to say thank you to all microbiologists who took part in our competition and also to our independent jury members – Professor, Dr. Lars Fieseler (ZHAW) and Professor, Dr. Mohammad Manafi (Medical University of Vienna). The aim was to encourage microbiologists to promote their work, and the entries were supposed to illustrate any microorganisms (living or dead) or a microbiologist in action at work.

The winning image will also be featured on the first Microbiology Focus in 2013 (Volume 5.1). Of course it will be accompanied by an interesting article from the winner, Rethish Raghu.

First Place

Inside a Ciliate's World

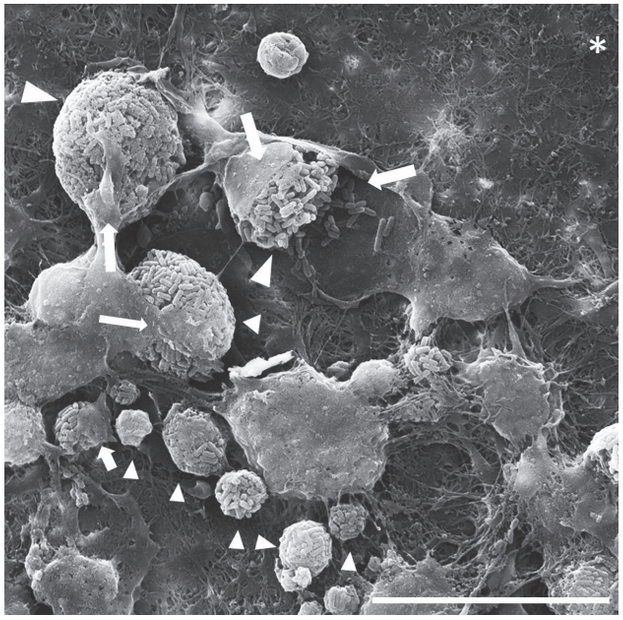


High magnification of the exterior of a *Colpoda* sp. ciliated protozoan cell (red). Cilia (green), many extracellular *Listeria monocytogenes* cells (brown), and co-culture debris (blue) are present. (Magnification: 20,000 ×; Instrument used: Philips XL30 Field Emission Scanning Electron Microscope at Adelaide Microscopy; Color added through Adobe® Photoshop® CS5).

Winner of an iPad®: Rethish Raghu
(University of Adelaide, Australia)

Second Place

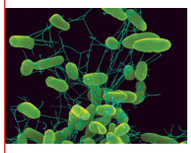
Listeria monocytogenes against Brain Macrophages



Listeria monocytogenes has a particular tropism for the central nervous system. To gain knowledge about the immune response elicited by *L. monocytogenes* in the brain, we used a rat ex-vivo organotypic nervous system culture as a model for *Listeria* infection. Brain sections were maintained alive for several weeks to study the infection process.

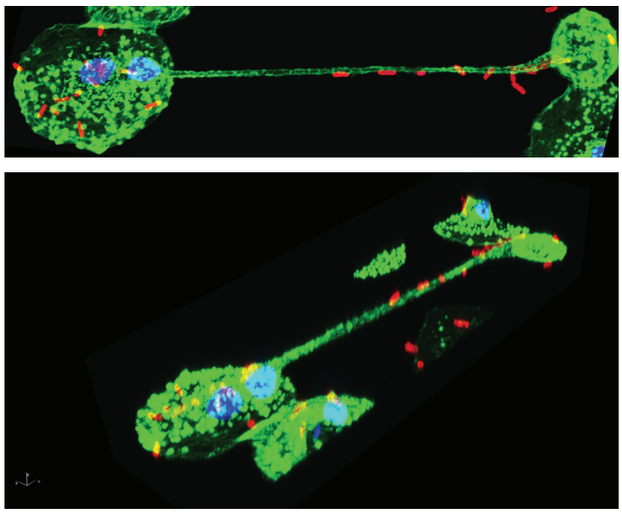
Scanning electron microscopy reveals how brain phagocytic cells (called brain macrophages or "microglia") are quickly recruited from deeper brain layers to the surface of the infected tissue. Picture shows the surface of the brain and a dead microglial cell fragmented into phagosomes of different sizes still filled with *L. monocytogenes* (arrowheads). New phagocitic cells arrive to engulf this dangerous cargo (arrows). Magnification: ×5,000. Scale bar: 20 µm.

Winner of a Swiss Army® knife: Ramos Vivas Jose
(Hospital Universitario Marqués de Valdecilla and Instituto de Formación e Investigación Marqués de Valdecilla)



Third Place

A Dangerous Bridge for *Serratia*



Fluorescence image with a 3D orthogonal view: *Serratia* spp. are widely distributed in nature. *Serratia marcescens* is the most common *Serratia* sp. associated with human disease, followed by strains of the *S. liquefaciens* complex: *S. liquefaciens*, *S. grimesii* and *S. proteamaculans*. The clinical significance of these species is largely unknown, because most clinical data refer to the *S. liquefaciens* group and do not differentiate the species therein.

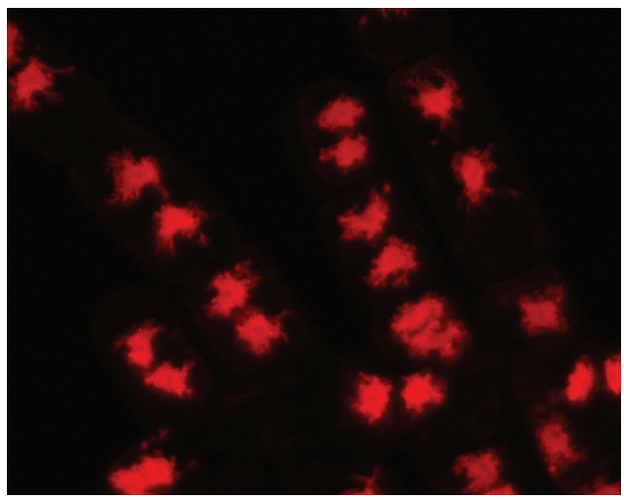
Macrophages play key roles in host defense by recognizing, engulfing, and killing microorganisms. We used several *in vitro* assays to investigate the interaction between *Serratia* and macrophages. The picture shows a Confocal Laser Scanning Micrograph (CLSM) of human peripheral blood monocytes differentiated to macrophages, and infected with a red fluorescent *Serratia liquefaciens*. A macrophage tries to capture bacteria by means of a long pseudopod. Bacteria seem to walk along this structure…

After fixation, cells were permeabilized with Triton® X-100. Atto-488 phalloidin (Sigma-Aldrich®), which binds polymerized F-actin, was used to identify actin filaments and fibers. Preparations were mounted in Fluoroshield-mounting medium containing DAPI (Sigma-Aldrich). (Series of optical sections were obtained with a Nikon® A1R confocal scanning laser microscope equipped with a Nikon A1 digital camera, and a 403nm, 488nm, 561nm lasers; Original Magnification: ×600)

Winner of an USB flash drive: Ramos Vivas Jose
(Hospital Universitario Marqués de Valdecilla and Instituto de Formación e Investigación Marqués de Valdecilla)

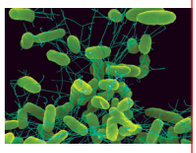
Fourth Place

Zygnema an Indicator for Freshwater



The filamentous green alga *Zygnema* (400 fold magnification) is an indicator for cold, clear and nutrient-poor freshwater. Each cell contains two star-shaped chloroplasts (flanking the cell nucleus) which show a red autofluorescence under UV illumination.

Winner of a laser pointer: Helmut Brandl
(University of Zurich)



Corynebacterium diphtheriae

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

Diagnosis of an Uncommon Potential Pathogen

Corynebacterium diphtheriae is a Gram-positive, aerobic, non-motile (no flagella), rod-shaped pathogen. It belongs to the group of a Actinobacteria (**Table 1**) and, is therefore, related to mycobacteria and actinomycetes. Natural sources for *Corynebacterium* are soil, water, plants and food products.

Four subspecies are known: *C. diphtheriae mitis*, *C. diphtheriae intermedius*, *C. diphtheriae gravis* and *C. diphtheriae belfanti*; all of them may be toxigenic. The toxin's origin from *Corynebacterium diphtheriae* is from a bacteriophage, specifically its DNA. The DNA expression depends on the iron concentration in the surrounding environment. In the presence of iron, the production of the toxin is stopped. The toxin is taken up into human cells and then cleaved into a protein containing an enzyme which inhibits RNA synthesis.

Diphtheria, the disease caused by the toxin, is an acute infectious disease primarily of the upper respiratory tract but occasionally also of the skin.

Kingdom	Bacteria
Phylum	Actinobacteria
Order	Actinomycetales
Suborder	Corynebacterineae
Family	Corynebacteriaceae
Genus	*Corynebacterium*

Table 1. Taxonomic classification

For the accurate identification of *C. diphtheria*, a Gram stain is performed to show Gram-positive organisms. Particular staining methods (Albert's and Ponder's stain, containing toluidine blue or Neisser's Methylene Blue stain) can be used to identify the highly pleomorphic organisms. The typical metachromatic granules can then be seen at the polar regions.

Figure 1: A photomicrograph of *Corynebacterium diphtheriae* taken from an 18 hour culture, using Albert's stain. (Source: CDC 1979)

To culture *Corynebacteria*, enriched media are utilized for the process; for example, Loeffler's medium is recommended. All *Corynebacteria* need biotin to grow and generally grow quite slowly. Other media that can be used for isolation and detection are Blood Agar, Tinsdale Agar, Cystine-Tellurite blood agar, Hoyle's Tellurite Agar, TSA and Nutrient Agar. To enhance growth, blood and serum may be used. For differentiation, the tellurite reaction can be employed, since all *Corynebacteria* (including *C. diphtheriae*) are able to reduce tellurite to metallic tellurium, which is then evidenced by the formation of brown to black colonies; for *C. diphtheriae*, a black halo can be seen around the colonies. In addition, potassium tellurite may serve as a selective substance. Currently, one of the best methods used to detect

Strain	Growth on Blood Agar	Catalase	Lactose*	Ribose*	β-Glu	β-Gur	Nitrate	Urease
C. diphtheriae gravis	non-haemolytic	+	–	+	+	+	+	–
C. diphtheriae mitis	Small zone of β-haemolysis	+	–	+	+	+	+	–
C. diphtheriae intermedius	Small zone of β-haemolysis	+	+	+	+	+	+	–
C. diphtheriae belfanti	Small zone of β-haemolysis	+	–	–	+	+	–	–

* = acid production; β-Glu = β-Glucosidase; β-Gur = β-Glucuronidase

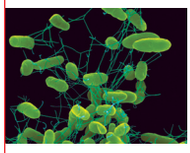
Table 2. Typical properties for *C. diphtheriae*

Corynebacteria employs a selective medium such as Tinsdale Agar with the addition of potassium tellurite. Tinsdale proposed a serum-cystine-thiosulfate-tellurite agar for the isolation and differentiation of *C. diphtheriae*. In this way, *C. diphtheriae* and diphtheroids could be differentiated. The modern formulation was modified by using Proteose Peptone, which improved the differential qualities and recovery of *C. diphtheriae*. The halo formation of *C. diphtheriae* on the medium is unique with one exception; colonies of *C. ulcerans* occasionally show a similar appearance, but exhibit a positive urease test.

For the biochemical testing for confirmation or differentiation of organisms, techniques applying the heamolytic reaction on Blood Agar, catalase test, nitrate and urease test are primarily employed. In addition, a number of other typical properties, some of which are listed in **Table 2**, may be applied in biochemical analyses.

Description	Cat. No.
Staining	
Neisser's Methylene Blue Solution	01363
Gram Staining Kit	77730
Media	
Hoyle's Tellurite Agar, Base – New	12282
Tinsdale Agar (Base)	89747
Columbia Agar	27688
Blood Agar (Base)	70133
Supplements	
Potassium tellurite Solution	17774
Tinsdale Supplement	73831
Tests	
Catalase Test	88597
Nitrate Reagent Disks Kit	51138
Nitrate Reduction Test	73426
Nitrate Reagent A	38497
Nitrate Reagent B	39441

Table 3: The most important reagents, tests and media from Sigma-Aldrich®



Corynebacterium diphtheriae

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