Microbiology Focus

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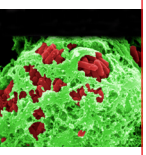
Fluka Analytical

Updates on *Listeria monocytogenes*

Cheese made from raw milk and raw sausage products are high-risk substances for *Listeria monocytogenes* infections. The Scanning Electron Micrograph shows a dead, blown-up phagocytic cell (green) that has lost large membrane areas due to a voracious appetite or to a massive intracellular proliferation of *L. monocytogenes* (red).
(Source: José Ramos Vivas, HUMV -IFIMAV, 2013)



Listeria Monocytogenes: Full Adherence

By Sara Remuzgo-Martínez, María Lázaro-Díez, Natalia Wasielewska, Ana Franco-González-de-Canales and Jose Ramos-Vivas,
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Listeria monocytogenes is a ubiquitous Gram-positive, motile, facultative intracellular bacterium, responsible for severe foodborne infections in humans. Listeriosis often occurs as a sporadic disease, but can also occur as large outbreaks with fatality rates of 25-30%. This organism can be introduced to food-processing environments through many routes and may become established on food-processing equipment. Identification and discriminatory monitoring of human and food strains can help to identify pathways for the distribution and harborage of *Listeria* spp. in food processing plants, which is important for their control. Despite this increased recognition, listeriosis is still an uncommon infection and may not immediately come to mind in the evaluation of the patient with sepsis, gastroenteritis or meningitis. In fact, the diagnosis of listeriosis may not be considered in the empirical treatment of bacterial meningitis before the organism is identified in blood or cerebrospinal fluid (CSF), which can lead to delays in diagnosis and inappropriate treatment with a poor outcome (Schlech and Acheson, 2000). A variety of conventional and rapid methods are available for the detection and identification of *L. monocytogenes* in food samples and specimens from animal and human listeriosis (Microbiology Focus 2.2, 2010). Aside from the biochemical reactions, Listeria spp. can be quickly confirmed as *L. monocytogenes* by using primers specific to the hemolysin (hlyA) or internalin A (inlA) genes unique to *L. monocytogenes* (Kushwaha and Muriana, 2009), and by other genomic or proteomic approaches.

The ability of the pathogen to survive at low temperatures, to colonize surfaces in the form of biofilm-like structures, and to resist various food-related stressors such as heavy metals and disinfectants is crucial for its persistence in the processing environment. According to Hall-Stoodley et al., biofilms can be defined as "a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface" (Hall-Stoodley et al., 2004). Microorganisms growing in biofilms are protected against cleaning and disinfection and are difficult to eradicate (Boyle et al., 2013) and therefore, the biofilm eventually constitutes a reservoir of dissemination and cross-contamination in foods. Significant differences have been reported regarding the ability of different *L. monocytogenes* isolates to form biofilms, but no clear correlation can be established with serovars or lineages (Renier et al., 2011). To gain knowledge about the adherence and biofilm formation in *L. monocytogenes*, we used different systems as models and several microscopy approaches. *L. monocytogenes* HUMV strain 3292 isolated from human placenta was grown in 8-well µ-chamber uncoated slides (Ibidi, Martinsried) without shaking. Unfixed planktonic cells were removed after 48h and bacterial viability within biofilms was determined by using a LIVE/DEAD bacterial viability kit. A series of optical sections were obtained with a Nikon A1R confocal laser scanning microscope and 488/561 nm excitation, 500-550/570-620nm emission filters were used. Images were captured at random with a ×20 Plan Apo 0.75 NA objective. Reconstructions of confocal sections were assembled using the NIS-Elements 3.2 software. **Figure 1** shows CLSM images of unfixed biofilms formed by L. monocytogenes HUMV-3292 and stained with LIVE/DEAD. **Figure 2** shows a scanning electron microphotography (SEM) of a biofilm formed by *L. monocytogenes* strain HUMV-4251 isolated from human cerebrospinal fluid at the Hospital Universitario Marqués de Valdecilla, Santander, Spain.

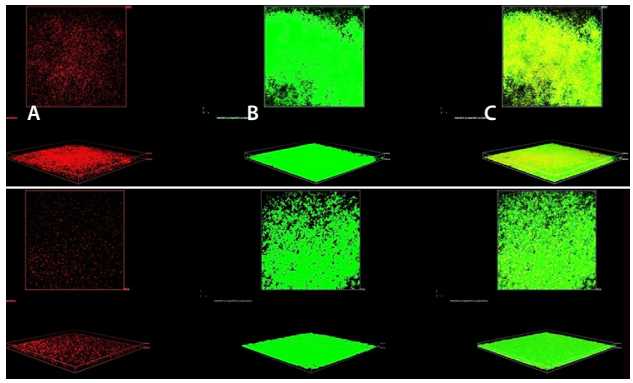

Figure 1: Maximum intensity projection CLSM images of unfixed biofilms formed by *L. monocytogenes* HUMV3292 at 37 ºC (upper panel) and room temperature (lower panel). A: dead cells (red); B: live cells (green); C: merge (Original magnification × 200. Scale bars: 10 µm)

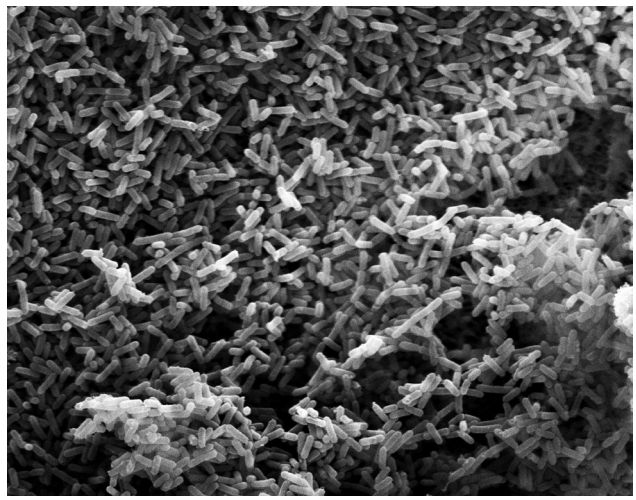

Figure 2: Scanning electron microscopy micrograph of a biofilm formed by *L. monocytogenes* HUMV-4251. Biofilms were fixed in ice-cold 3% glutaraldehyde for 20 min at 4 °C. Samples were then dehydrated in a graded ethanol series, dried by the critical point method, coated with gold in a fine coat ion sputter JFC-1100 (JEOL) and observed with an Inspect S microscope (FEI Company) working at 25 KV. Original magnification × 5000.

L. monocytogenes is also a bacillus with a high tropism for the human central nervous system (CNS) (Clauss and Lorber, 2008) and has been used as a model intracellular pathogen to study basic aspects of innate and acquired immunity (Remuzgo-Martínez et al., 2013a,b). In vitro data and in vivo clinical experience suggest that a combination of ampicillin and an

aminoglycoside is the favored treatment of invasive listeriosis. Also, vancomycin in combination with an aminoglycoside has been successfully used as treatment for penicillin-allergic patients with listeriosis. All strains of *L. monocytogenes* are uniformly resistant to cephalosporin antibiotics and recent data suggest an emerging antimicrobial resistance in Listeria spp. and emphasize the need for further genotypic characterization of antibiotic resistance in this pathogen (Lungu et al., 2011).

In spite of the great clinical importance of *L. monocytogenes* infections in the CNS, knowledge about the interactions between the bacteria and neurons is still scarce. We have performed some experiments to study the adherence of several L. monocytogenes strains to CNS cells. Examples are shown in **Figures 3** and **4**. Experimental studies are in progress in our lab to show different aspects of the adherence of *L. monocytogenes* to inert surfaces and to CNS phagocytic and non-phagocytic cells.

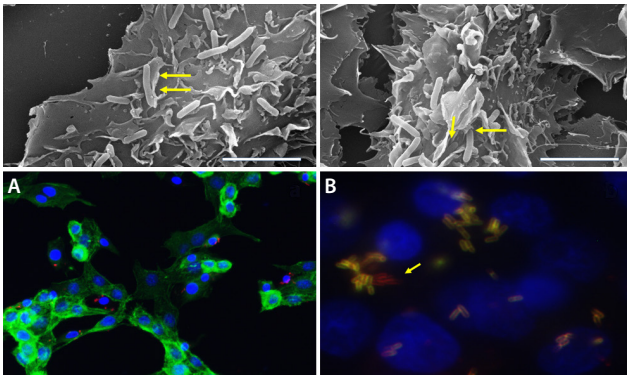

Figure 3: Human cells (cell line U-87, astrocytoma cell line derived from human malignant glioma) infected for 45 mins with L. monocytogenes strain HUMV-4251 and stained by immunofluorescence, showing the typical zipper mechanism for cell adherence and invasion (arrows, upper panel). (a) U-87 cells stained with Atto 488-phalloidin showing intracellular *L. monocytogenes* (in red). (b) In merged images, extracellular bacteria are shown in orange or yellow and intracellular bacteria in red. Arrow indicates some intracellular bacteria. DAPI-stained nuclei are shown in blue. Micrographs were originally captured at ×15000 (SEM), ×400 magnification (a) or ×1000 magnification (b). Scale bars: 5 µm.

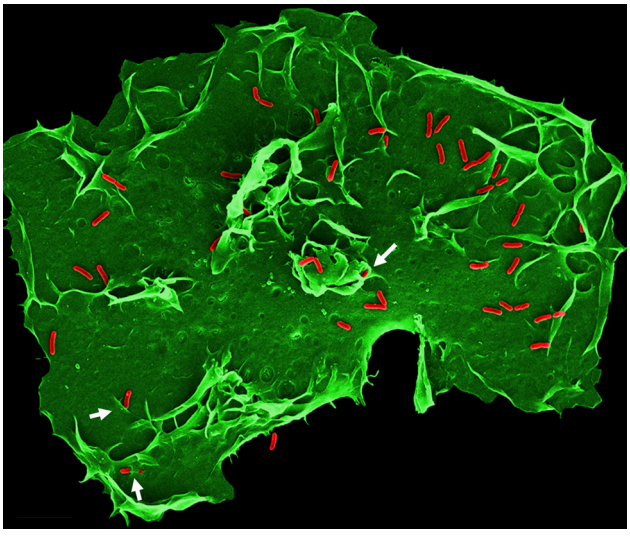

Figure 4: Pseudocolored SEM microphotography of a rat primary microglial cell infected with *L. monocytogenes* strain HUMV-4251, showing the typical zipper mechanism for cell adherence and invasion (arrows). Micrograph was originally captured at × 5000 magnification. Scale bar: 5 µm.

The factors required for biofilm formation in *L. monocytogenes* are still relatively unknown. The results gained during our study indicate that factors involved in the attachment of *L. monocytogenes* to surfaces can be strain-specific. Since attachment is also the first step in initiating cellular infection, it would be interesting to see in future research if strong adherence to abiotic surfaces correlates with enhanced cellular attachment in tissue culture and organotypic tissue assays.

References:

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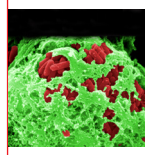
9. Schlech, W.F. and Acheson, D. Foodborne Listeriosis. *Clin Infect Dis*. 2000, 31: 770-75.

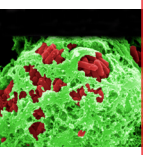
Did you know…

Sigma-Aldrich counts Listeria bacteria and puts them in a disc?

Under the name Vitroids™, Sigma-Aldrich sells Certified Reference Materials, a unique microorganism standard with the lowest standard deviation and highest stability (**sigma-aldrich.com/vitroids**)

Figure 1: Vitroids™





Listeria Detection

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To avoid *Listeria infections*, it is important to regulate high-risk food.

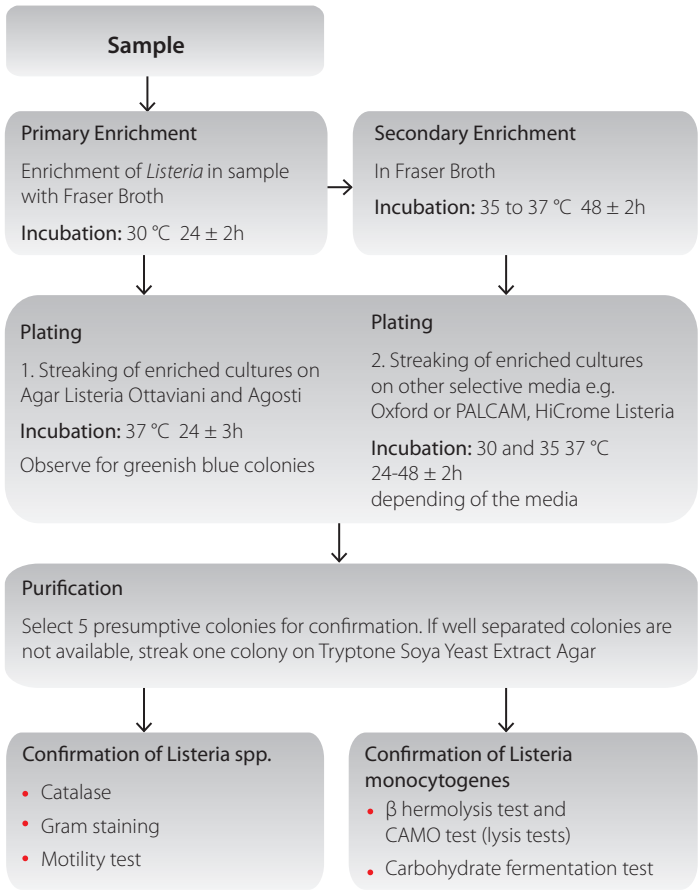
Frequently we have read about Listeria cases where food is the presumptive or proven source of contamination. In the worst cases, infection results in spontaneous abortions as well as in the death of newborns and people in high-risk groups. In addition, the trend toward increased consumption of ready to eat food, prolonged shelf life of food and gentle processes for food preparation, have contributed to the increase in *Listeria* cases over the last decade. Making food safe, therefore, is an important responsibility.

Therefore ISO (11290-1 and 11290-2) recommends a four-step process: enrichment, identification, isolation and confirmation. A flow chart of the processes appears in **Figures 1** and **2**. Currently, diverse new methods are available such as the in situ hybridization from Sigma-Aldrich called HybriScan™.

For more details visit
sigma-aldrich.com/hybriscan

Tools used to detect *Listeria*, especially *Listeria monocytogenes*, are selective media containing phenyl ethanol and a high concentration of lithium chloride and sodium chloride and/or antibiotics such as moxolactam, nalidixic acid, polymyxin B sulphate, ceftazidime, amphotericin B, acriflavine, cycloheximide, colistin sulphate, cefotetan and fosfomycin. An additional smart solution is the use of chromogenic media. There are various commercially available chromogenic media like the Agar Listeria Ottavani and Agosti, and most of them use the following systems for differentiation:

- Detection of β-glucosidase activity (by X-glu = 5-bromo-4-chloro-3indolyl-β-D-glucopyranoside) and also Rhamnose fermentation (by indicator phenol red) on a selective media. *Listeria monocytogenes* and *Listeria innocua* result in blue colonies with yellow background, while Listeria ivanovii shows only blue colonies.
- Screen for the presence of β-glucosidase (by X-glu) and phosphatidylinositol specific phospholipase C on a selective media. *Listeria monocytogenes* and Listeria ivanovii result in greenish-blue colonies with an opaque halo, while *Listeria innocua* shows only greenish-blue colonies (recommended by ISO 11209-2).



Products for Enrichment Steps

Fraser Broth, Base (**Fluka 69198**)
Fraser Selective Supplement (**Fluka 18038**)
Fraser Supplement (**Fluka 90836**)

Products for Plating

Agar Listeria Ottaviani and Agosti (**Fluka 7708**)
Listeria mono Enrichment Supplement I (**Fluka 03708**)
Listeria mono Enrichment Supplement II (**Fluka 92301**)
Listeria mono Enrichment Supplement III (**Fluka 91603**)
Oxford Agar (**Fluka 75805**)
Oxford-Listeria Selective Supplement (**Fluka 75806**)
PALCAM Listeria Selective Agar (**Fluka 75977**)
PALCAM Listeria Selective Supplement (**Fluka 03396**)
HiCrome™ Listeria Agar Base, modified (**Fluka 53707**)
HiCrome™ Listeria Selective Supplement (**Fluka 59688**)

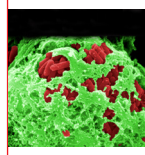
Purification Medium

Tryptone Soya Yeast Extract Agar (**Fluka 93395**)

Products for Confirmation

Catalase Test (**Fluka 88597**)
Gram Staining Kit (**Fluka 77730**)
Listeria Motility Medium (**Fluka 55265**)
Carbohydrate Consumption Broth (**Fluka 07410**)
Blood Agar Base No. 2 (**Sigma B1676**)

Figure 1: ISO 11290-1:1996 / A1:2004 - Detection method of *Listeria monocytogenes*.



Typical biochemical tests and properties to confirm, differentiate and identify Listeria are catalase test (positive), oxidase test (negative), fermentation of carbohydrates, hydrolysis of esculin and sodium hippurate, methyl red, ammonia production from arginine, hydrogen sulfide (negative), indole (negative), nitrate reductase (negative), gelatin liquefaction (negative), hydrolysis of starch (negative) and urea hydrolysis (negative). Specific differentiation of *L. monocytogenes* is done by β-hemolysis test (positive) then followed by detection of carbohydrate fermentation ability (Rhamnose +, Xylose -). Further possibilities are methyl α-D-mannopyranoside and CAMP-test. In the CAMP-test, some *Listeria species* show the ability to enhance the haemolysis of *Staphylococcus aureus*.

More enrichment, selective and differential media can be found under
sigma-aldrich.com/listeria

Did you know…

To protect food from *Listeria* special bacteriophages could be taken

The first products with specific bacteriophages are on the market to control *Listeria monocytogenes* in food and in f ood processing.

Figure 1: Eating a fresh cheese product.

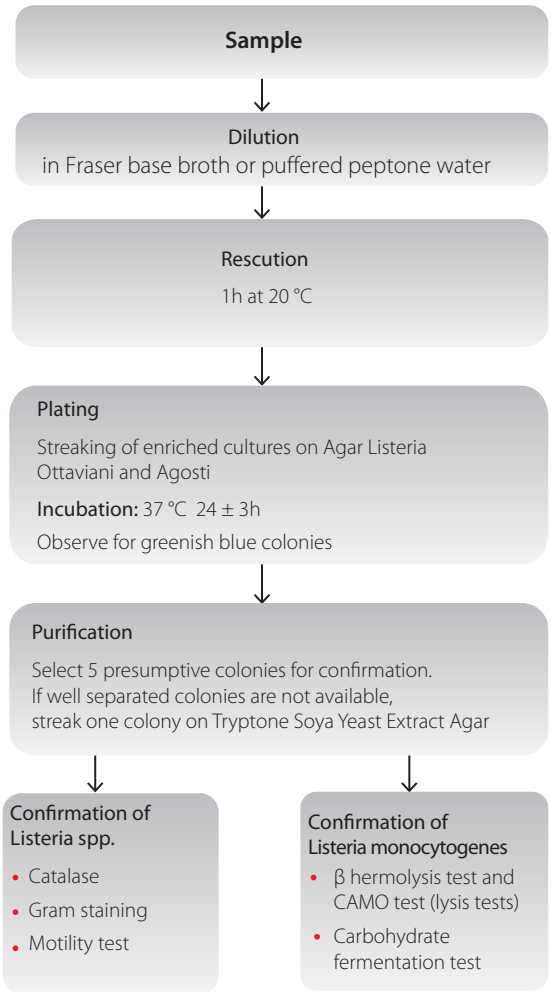


Figure 2: ISO 11290-2:1998 / A1:2004 - Enumeration of *Listeria monocytogenes*.

Products for Enrichment Steps

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Fraser Selective Supplement (**Fluka 18038**)
Fraser Supplement (**Fluka 90836**)

Products for Plating

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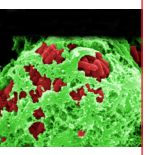

Membranes for Microbial Rapid Identification

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Classical plates are still used today as first steps in modern microbiology. Smart, inexpensive membranes can help to identify organisms within 1 to 4 hours.

These membranes, with 70 mm diameter, are for economical and rapid identification and confirmation of microorganisms in water, food, environmental and clinical samples. They find their application in various sectors in the food and dairy industry, water industry, pharmaceutical laboratory testing, cosmetic industry, environmental and sanitary testing, clinical diagnostics, etc.

After the routine inoculation and isolation technique, the membranes enable direct identification just by placing them on the agar plate. Common technologies, known from classical and innovative media such as indicators, chromogenic and fluorogenic substrates, are used for the differentiation system.

Method Workflow

Inoculation and Isolation: Inoculate the organisms from sample on any general purpose media, nutrient agar, tryptic soy agar, plate count agar, etc.

Adopt any of surface plating methods, such as spread plate method, quadrant (four or five) streak pattern or T streak method so as to obtain isolated colonies from inoculums.

Incubation: Incubate at 35-37 °C for 18-24 hours.

Replication: For replication technique, place the membrane on the surface of agar plate. Perform this step for a maximum of 30 seconds to 1 minute. Mark the corresponding orientation of paper.

Identification: Incubate the replicated identification membrane in empty, sterile Petri dish at 35-37 °C for 1-4 hours, or if desired membrane can be placed on dry lid of same plate, incubate in inverted position (*if lid has moisture, wipe it with sterile cotton). Alternately, the membrane may be kept for incubation on growth media at 35-37 °C. Observe for development of color and interpret results

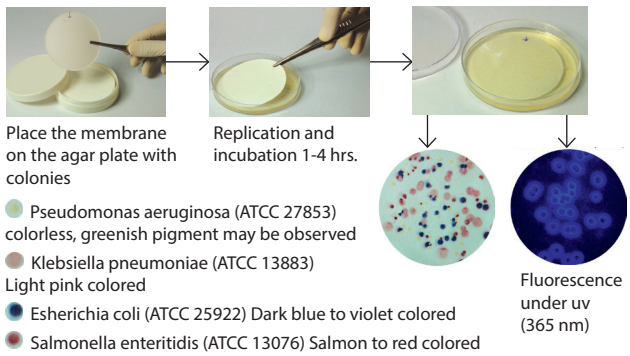

Figure 1: Workflow of ID Membranes.

Cat. No.	Membrane	Product Description
78039	Bacillus ID Membrane	For rapid detection and differentiation between various species of Bacillus such as B. subtilis (yellowish green to green), B. cereus (light blue), B. thuriangiensis (light blue), B. megaterium, B. coagulans, B. pumilus from food, meat, fish, cosmetic and pharmaceutical preparations
01433	Biochemical Esculin ID Membrane	For rapid detection of Group D Streptococci (black) from food, dairy, water samples and pharmaceutical products, etc.
19933	Differential Coli - E. coli ID Membrane	For rapid detection of *E. coli* (dark blue to violet), Klebsiella (light pink), Pseudomonas (colorless) and Salmonella (salmon to red) species in food and environmental samples
66964	Differential ID Membrane	For rapid differentiation of Lactose fermenting (dark pink) and Lactose (colorless) non-fermenting enteric bacteria from water, food, dairy products, cosmetics, pharmaceutical preparations, etc.
73257	DNase ID Membrane	For rapid detection of deoxyribonuclease (DNase) activity of bacteria, especially for identification of pathogenic Staphylococci (pink zone around the colony, DNase positive)
03719	Dual Confirmation of *E. coli* ID Membrane	For rapid detection and confirmation of Escherichia coli (blue and positive fluorescence) in water and food samples, based on chromogenic and fluorogenic methods
93009	*E. coli* Chromogenic ID Membrane	For rapid detection and confirmation of *Escherichia coli* (blue) in water and food samples
06722	*E. coli* Fluorogenic ID Membrane	For rapid detection and confirmation of *Escherichia coli* (fluorescence positive) in water and food samples on the basis of fluorogenic emission at 365 nm
51161	Pseudomonas ID Membrane	For rapid detection of Pseudomonas aeruginosa (fluorescence positive) from clinical and non-clinical specimens
68122	Salmonella ID Membrane	For rapid detection of Salmonella species (light purple) from coliforms (blue = *E. coli*, others are colorless)
77396	Total Coliform ID Membrane	For qualitative detection of coliforms from water, pharmaceutical preparations, dairy and food products. (*Escherichia coli* = dark blue; Enterobacter cloacae = salmon to red; Citrobacter freundii = salmon to red; Klebsiella pneumonia = light pink)
39187	Universal Environmental ID Membrane	For rapid detection of Pseudomonas (colorless, greenish pigment is observed), Enterococcus (blue – blue green, small), *E. coli* (pink-purple), Staph. aureus (golden yellow) and Salmonella (colorless) species, etc. from environmental samples, samples of clinical origin such as nosocomial samples
15713	Universal Food Pathogen ID Membrane	For rapid detection of food pathogens such as *E. coli* (purple), *E. coli* O157:H7 (purple-pink), Staphylococcus aureus (golden yellow), Salmonella (colorless), Bacillus (light green, big), Listeria (blue- green) and Shigella (colorless) species, etc. from various food, dairy, fish, and meat products
00446	Universal Microbial Limit Test Membrane	Recommended for detection of pathogenic microorganisms such as *E. coli* (pink-purple), S. aureus (green to bluish-green), P. aeruginosa (colorless), Bacillus (colorless) and Salmonella (colorless) species from pharmaceutical preparations, raw materials, cosmetic samples, etc.
30374	UTI ID Membrane (Urinary Tract Infections ID Membrane)	For rapid detection and confirmation of microorganisms mainly causing urinary tract infection, e.g., *E. coli* (pink-purple), Proteus (light brown), Klebsiella (blue to purple, mucoid), Pseudomonas (colorless), S. aureus (golden yellow), and Enterococcus species (blue – blue green, small)
5687	Glucose Fermentation Membrane	For rapid detection of carbohydrate fermenting organisms from mixed flora where fermenting organisms will exhibit yellow color
52284	Lactose Fermentation Membrane	
39406	Mannitol Fermentation Membrane	
41473	Sucrose Fermentation Membrane	
92601	Xylose Fermentation Membrane	

Figure 2: Range of ID Membranes (more details and images can be found on the web)

Klebsiella

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Klebsiella is a genus which contains opportunistic pathogens.

Klebsiella is a Gram-negative, rod shaped and non-motile bacteria belonging to the family of Enterobacteriaceae. They tend to be rounder and thicker than the other Enterobacteriaceae. This genus got their name from the German/Swiss bacteriologist Edwin Klebs (1834-1913). In the presence of oxygen, *Klebsiella* has an oxidative metabolism using organic compounds and ending in carbon dioxide and water. If no oxygen is present, the organism switches to the end product 2,3-butanediol, carbon dioxide and different organic acids, so it is a facultative anaerobe. In comparison to other members of the Enterobacteriaceae family, like Escherichia and Salmonella, which produce lot of acetic acid, lactic acid and succinic acid, the butanediol fermentation leads only to small amounts of acid. This characteristic property can be used for the differentiation. Therefore, the Voges Proskauer test, a reagent which detects acetoin production, an intermediate of the butanediol fermentation, can be used (see biochemical identification and differentiation in **Table 2**).

Kingdom: Bacteria
Order: Enterobacteriales
Phylum: Proteobacteria
Family: Enterobacteriaceae
Class: Gammaproteobacteria
Genus: Klebsiella

Klebsiella species grow well on standard media like Nutrient Broth No. 1, and most strains can survive with citrate and glucose as the only carbon source and ammonia as the sole nitrogen source. They are able to ferment most carbohydrates. Optimum growth is found at a pH of 7.2 and a temperature of 35-37 °C. It poses a polysaccharide based capsule, which makes it resistant to phagocytosis.[1,2] This is also the target for serological tests, and it is also visible as typical slime layer on the colonies. All species are resistant to penicillin.

Did you know…

Klebsiella spp. increasingly developed an antimicrobial resistance?

Klebsiella belong to the family of Enterobacteriaceae and are a normal part of the human intestine. They can become highly resistant to antibiotics, and produce an enzyme called carbapenemase, which can destroy the carbapenems. Therefore, this group of antibiotics does not work for treatment.

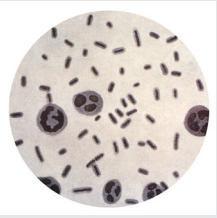


Figure 1: Photomicrographic view of a Hiss capsule-stained culture specimen revealing the presence of numerous *Klebsiella pneumonia*. (Source: CDC, 1979)

Useful Media

A chromogenic media like the HiCrome™ *Klebsiella* Selective Agar can help to easily detect Klebsiella species as mucoid purple-magenta colonies. The chromogenic substrate incorporated in the media is cleaved specifically by *Klebsiella* species to produce purple-magenta colored colonies (see **Figure 2**). Most of the frequently encountered Gram-negative fecal contaminants are inhibited in this media using carbenicillin in the supplement. Bile salts mixture and sodium lauryl sulfate inhibit Gram-positive accompanying flora.

M-FC Agar modified for Klebsiella is a modification of M-FC Agar to detect E. coli and other coliforms. The medium is modified by replacing lactose with inositol and adding carbenicillin. Inositol as the fermentable carbohydrate makes the medium more selective as inositol can't be used by many other organisms. Synthetic detergent inhibits Gram-positive organisms and replaces the originally used bile salt. Carbenicillin inhibits the growth of Gram-negative organisms. Occasional false positive occurrences are caused by *Enterobacter* species (e.g., *Enterobacter aerogenes* gives pink or pale yellow colonies). Klebsiella colonies appear deep blue to blue grey due to aniline blue present in the medium, indicating fermentation of inositol.

In nature, *Klebsiella* can be found in soil, in water and in cereals. They can also be part of the natural flora in the human nose, mouth and intestinal tract.[3]

Clinically relevant species are *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Klebsiella granulomatis*. *Klebsiella granulomatis* is responsible for granuloma inguinale, a sexually transmitted disease characterized by ulcerative genital lesions. The other Klebsiella organisms are reported to lead to a wide range of diseases like pneumonia, urinary tract infections, septicemia, meningitis, diarrhea, and soft tissue infections.[3,4]

Media	Cat. No.
HiCrome™ *Klebsiella* Selective Agar Base **Supplement:** 2 vl/l Klebsiella Selective Supplement (**Fluka 15821**)	90925
m-FC Agar modified for Klebsiella, Vegitone **Supplements:** 100 mg/l Rosolic acid (**Fluka 83910**) 50 mg/l Carbenicillin (**Fluka 21800**)	07012

Table 1: Klebsiella identification and differentiation by biochemical methods.

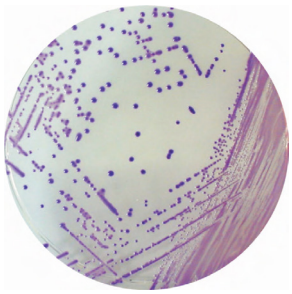
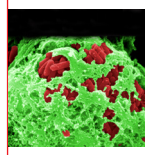


Figure 2: HiCrome™ Klebsiella Selective Agar Base (Klebsiella pneumonia as mucoid, purple-magenta colonies)

Continued on following page



Tests		Klebsiella oxytoca	Klebsiella pneumoniae	Products from Sigma-Aldrich®	Cat. No.
	Methyl Red	-	-	Methyl Red Solution	08714
	Voges-Proskauer	+	+	Methyl Red Voges Proskauer Saline Broth	69150
				O'Meara's Reagent	07689
				Barritt's Reagent A	29333
				Barritt's Reagent B	39442
	Citrate	+	+	Simmons Citrate Agar	85463
	Motility	-	-	Motility Test Medium	M1053
				MIO Medium	M1428
	Indole	+	-	Kovac's Reagent for Indoles	67309
				(with n-butanol)	60983
				Kovac's Reagent Strips	78719
	Arginin	-	-	—	—
	Lysine	+	+	L-Lysine Decarboxylation Medium	41932
				Lysine Decarboxylase Broth	66304
	Ornithine	-	-	Ornithine Decarboxylase Broth	O5386
				MIO Medium	M1428
	Phenyl deaminase	-	-	Malonate Phenylalanine Broth	63286
				Phenylalanine Agar	78052
TSI/Kligler	Gas	+	+/-	Kligler Agar	60787
	H2S	-	-	Triple Sugar Iron Agar (acc. to ISO)	92499
	Butt	A	A	Triple Sugar Iron Agar	44940
	Slant surface	A	A	—	—
	DNase (25 °C)	-	-	Deoxyribonuclease Test Agar	70136
				(with Tryptose)	30787
				DNase Test Agar with Toluidine Blue	D2560
				DNase ID Membrane	73257
	Gelatinase (22 °C)	-	-	DEV Gelatin Agar	31433
				Nutrient Gelatin	70151
	Urea	+	+	Christensen's Urea Agar	27048
				Urea Agar Base (Christensen)	U1757
				Urea Broth	51463
	Malonate	+	+	Malonate Broth	M8802
				Malonate Phenylalanine Broth	63286
	ONPG	+	+	ONPG Disks	49940
	Adonitol	+	+	Adonitol Disks	55876
	Arabinose	+	+	Arabinose Disks	80372
	Lactose	+	+	Lactose Broth	94792
				Lactose Disks	28816
				Lactose Fermentation Membrane	52284
	Sorbitol	+	+	Sorbitol Disks	93998
	Sucrose	+	+	Sucrose Disks	94309
				Sucrose Fermentation Membrane	41473
Growth	5 °C	-	-	Nutrient Broth No. 1	70122
	10 °C	-	+		
	41 °C	+	+		

Table 2: *Klebsiella* identification and differentiation by biochemical methods (A = acid production, ONPG = ortho-Nitrophenyl-β-galactoside)

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4. Podschun, R.; Ullmann, U. Klebsiella spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors, *Clin Microbiol Rev*, 1998,11(4): 589–603 .

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