Microbiology Focus

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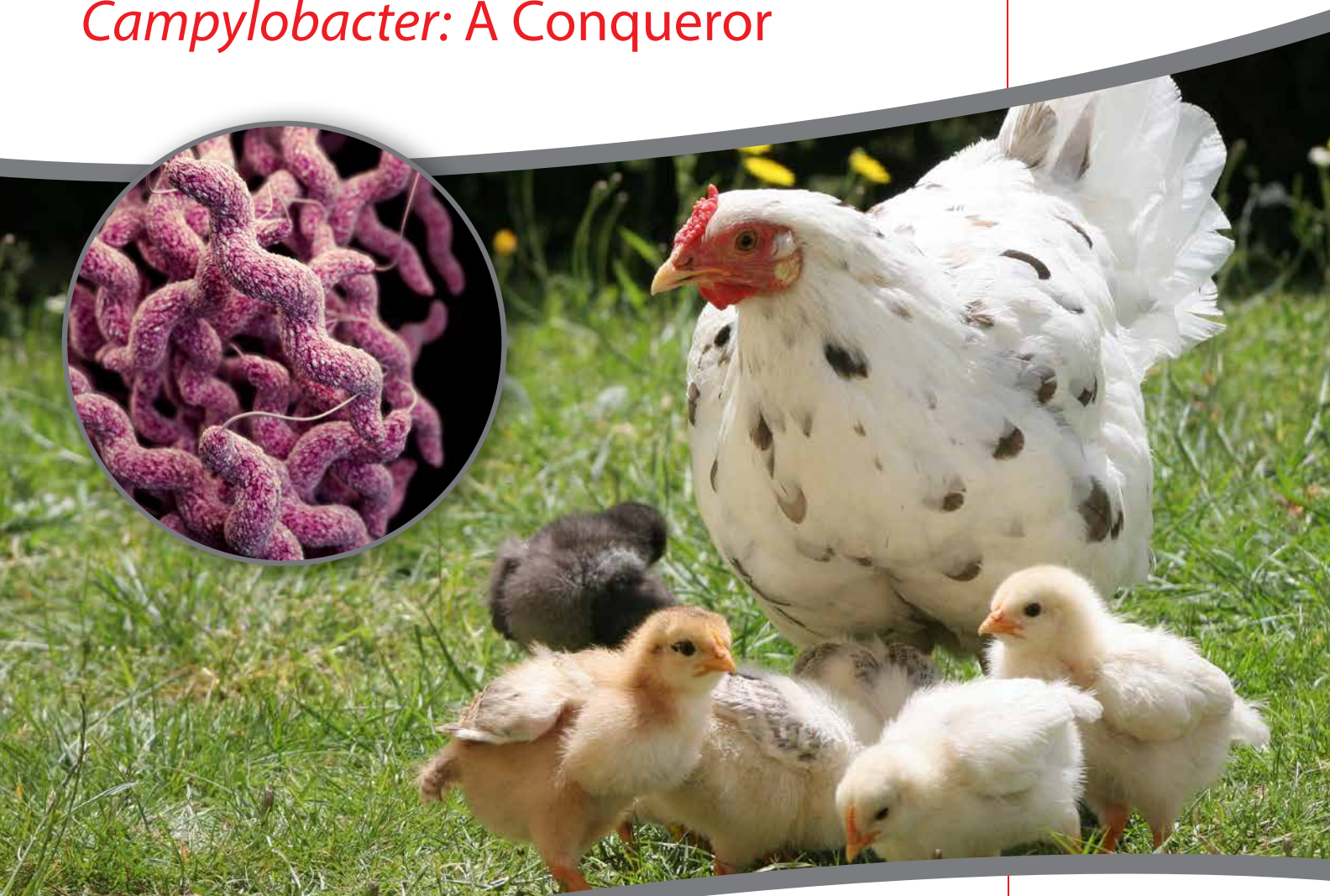
Campylobacter: A Conqueror



For many years, *Campylobacter* spp. had been known to cause diseases in animals like chickens, but beginning in the early 1980s, it was recognized as a cause of enteritis in humans. Scanning electron micrographic image of drug resistant *Campylobacter* bacteria. (Source: CDC / Melissa Brower 2013)

Campylobacter: A Conqueror

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Detection and differentiation of *Campylobacter* bacteria is still a tricky issue today.

Campylobacteriosis is currently one of the most common bacterial infections in human, and is often a foodborne illness. The usual symptoms are diarrhea, fever, cramping, abdominal pain and fever. In rare cases, *Campylobacter* spread to the bloodstream and cause a serious life-threatening infection.[3] The disease is usually self-limiting after 2–7 days; however, in some cases, particularly in the very young, elderly or immunocompromised, antibiotic treatment may be required. In more severe cases, hospitalization may result.

The two species, *Campylobacter jejuni* and *C. coli*, are responsible for the majority of human foodborne campylobacteriosis. There are other pathogen species – *C. lari, C. fetus* (infect reproductive tract), *C. upsaliensis* and very seldom *C. helveticus* which can also cause diarrheal illness.[1,2,3,7]

Campylobacter are ubiquitous and often found in domestic animals. Because of this, they are frequently found in the environment and on many raw foods of plant and animal origin. A very high concentration of *Campylobacter* can be found on raw poultry meat.[4] The possible carriers are wild birds which occasionally visit hen houses to steal feed and leave their feces on the ground. For young children playing outside, this can also be the direct source of infection.[8,9]

Did you know…

Supplementation of Bolton broth with triclosan improves detection of *Campylobacter jejuni* and *Campylobacter coli*?

Using this supplemented broth, Korean scientists were able to find a significantly higher number of *Campylobacter jejuni* and *Campylobacter coli* positive samples than they did using the normally recommended Bolton broth formulation. At the same time, the predominant competing flora was also significantly eliminated.

Figure 1: *Campylobacter* Bacteria
(Source: Jung-Whan Chon, et al., Supplementation of Bolton broth with triclosan improves detection of *Campylobacter jejuni* and *Campylobacter coli* in chicken carcass rinse, International Journal of Food Microbiology, Volume 181 (2014))

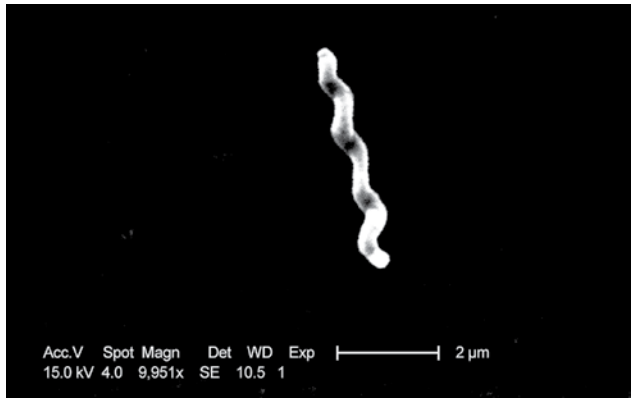

Figure 2: Scanning electron micrograph: *Campylobacter jejuni*, magnified 9,951x.
(Source: CDC/ Dr. Patricia Fields, Dr. Collette Fitzgerald 2004)

In most cases, the diarrhea results from the consumption of contaminated food. Poultry is a major food source of infection, and infection can occur through the consumption of undercooked or raw poultry meat or through cross-contamination of other food products. The symptoms of the disease can occur up to two weeks after consuming the contaminated food.[5]

Campylobacter are able to propel themselves through the mucous layer on top of the epithelial cells. Adhesion to the surface of the epithelial cells, followed by invasion into the cells, is also thought to occur before the onset of diarrhea. The production of the cytolethal distending toxin (Cdt) is the final stage prior to the start of symptoms. *C. jejuni*, for example, produce only Cdt A, B, and C, which seems to be important for cell cycle control and induction of host cell apoptosis, and has been recognized as a major pathogenicity-associated factor.[7]

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Epsilonproteobacteria
Order: Campylobacterales
Family: Campylobacteraceae
Genus: *Campylobacter*

Campylobacter are Gram-negative, spiral, rod shaped and motile bacteria with uni- or bi-polar flagella (see **Figure 2**). They are microaerophilic, requiring oxygen levels between 2 and 10% for growth. The size of a cell is roughly 0.2–0.8 x 0.5–5 µm and, interestingly, in culture they can undergo morphological change from spiral to spherical shape. *C. jejuni* and *C. coli* are classed as thermophilic *campylobacters* as they have an optimum temperature for growth of 42 °C. Most species, specially the pathogenic ones, are catalase- and oxidase-positive,[6] with the exception of the catalase-negative *C. sputorum, C. concisus, C. mucosalis* and *C. helveticus*. The metabolism of *Campylobacter* is chemoorganotrophic, with amino acids and intermediates of the citric acid cycle serving as energy sources; typical carbohydrates cannot be used. *Campylobacter* reduce nitrate to nitrite, obtaining oxygen for their metabolism by this pathway. These distinctive metabolic reactions can be used for the differentiation and identification of *Campylobacter* species (**Table 1**). Commercially available tests from Sigma-Aldrich appear in **Table 2**.

Species		*C. jejuni*		*C. coli*	*C. lari*	*C. fetus*	
Subspecies		*jejuni*	*doylei*			*fetus*	*veneralis*
Growth at	25 °C	no	no	no	no	yes	yes
	37 °C	yes	yes	yes	yes	yes	yes
	42 °C	yes	no	yes	yes	partial	no
Oxidase		yes	yes	yes	yes	yes	yes
Catalase		yes	yes	yes	yes	yes	yes
Nitrate Reduction		yes	no	yes	yes	yes	yes
Hippurate Hydrolysis		yes	yes	no	no	no	no
Indoxyl Acetate Hydrolysis		yes	yes	yes	no	no	no
H_2S Production		no	no	no	yes	yes	no
Antibiotic Sensitivity	Nalidixic acid	S	S	S	R/(S)	R	R
	Cephalotin	R	S	R	R	S	S
	Penicillin	R	R	R	R	R	S
	TTC*	R	R	R	S	S	S

Table 1: Table of differentiating characteristics of *Campylobacter* species and subspecies
* triphenyltetrazolium chloride

Campylobacter Test	Brand	Cat. No.
Catalase Test	Fluka	**88597**
Aminopeptidase Test (detection of Gram-negative microrganisms)	Fluka	**75554**
Gram Staining Kit	Fluka	**77730**
Hippurate Disks	Fluka	**40405**
Hydrogen Sulfide Test Strips	Fluka	**06728**
Indoxyl Strips	Fluka	**04739**
Nitrate Reagent A	Fluka	**38497**
Nitrate Reagent B	Fluka	**39441**
Nitrate Reagent Disks	Fluka	**08086**
Oxidase Test	Fluka	**70439**
Oxidase Strips	Fluka	**40560**
Oxidase Reagent acc. Gaby-Hadley A	Fluka	**07345**
Oxidase Reagent acc. Gaby-Hadley B	Fluka	**07817**
Oxidase Reagent acc. Gordon-McLeod	Fluka	**18502**

Table 2: Sigma-Aldrich tests for identification and differentiation of *Campylobacter*

Campylobacter are generally very fastidious microorganisms, can often be difficult to isolate, and grow only on complex media that have been amended with diverse essential amino acids and supplements, such as pyruvate, α-ketoglutarate, hemin, formate and other essential ions. For selective isolation of *Campylobacter*, the growth media can be supplemented with antibiotics such as cefoperazone, vancomycin, trimethoprim, amphotericin, cycloheximide, rifampicin, cefsulodin and polymyxin B sulfate. Typical agars and broths used for the detection, identification, differentiation, enumeration and cultivation of *Campylobacter* are listed in **Table 3**.

The current method used for detection of campylobacters from foodstuffs is the EN/ISO 10272-1 method (see **Figures 3 and 4**). This method relies on homogenization of a known amount of material in Bolton Broth (BB) with selective supplement and 5% lysed horse blood microaerobically at 41.5 °C for 40–48 hours. Aerobic incubation during enrichment was not very successful in isolation of the organism; therefore, microaerobic enrichment was suggested as the preferred method (Moran et al. 2009). After incubation, a certain amount of the enrichment broth is then plated onto modified charcoal cefoperazone deoxycholate agar (mCCDA, see **Table 3**) with the addition of a selective

Media	ISO	Cat. No.
Nonselective Media		
CASO Agar		**22095**
CASO Broth		**22098**
Columbia Blood Agar	10272-1:2006, 10272-2:2006	**27688**
Mueller Hinton Agar	10272-1:2006	**70191**
Nutrient Broth No. 5		**78104**
Tryptic Soy Agar		**22091**
Tryptic Soy Agar (ready to pour)		**79872**
Tryptic Soy Agar, Vegitone		**14432**
Tryptic Soy Broth		**43592**
Tryptic Soy Broth		**22092**
Tryptic Soy Broth No. 2		**51228**
Tryptic Soy Broth, Vegitone		**41298**
***Campylobacter* Selective Media**		
Blood Free Campylobacter Broth (Modified CCD - Preston Broth) CCD Selective Supplement		**59751** **77093**
Blood Free *Campylobacter* Selectivity Agar Base (mCCD Agar) Blood Free *Campylobacter* Medium Selective Supplement	10272-1:2006, 10272-2:2006	**B2426** **74807**
Bolton Broth Base Bolton Selective Supplement	10272-1:2006	**67454** **40568**
Brucella Broth Base Additives acc. data sheet	10272-1:2006 10272-2:2006	**B3051**
Campylobacter Selective Agar (Base) Park and Sanders Selective Supplement I Park and Sanders Selective Supplement II		**21378** **17191** **17194**
Karmali *Campylobacter* Agar (Base) *Campylobacter* Selective Supplement		**17152** **17780**
Nonselective Media for Biochemical Differentiation		
Blood Agar Base No. 2		**B1676**
Hippurate Broth*		**53275**
OF Test Nutrient Agar*		**75315**
Selective Media for Biochemical Differentiation		
Mac Conkey Agar No. 1		**70143**

Table 3: Typical Sigma-Aldrich media for *Campylobacter*
* Not sold in U.S.A.

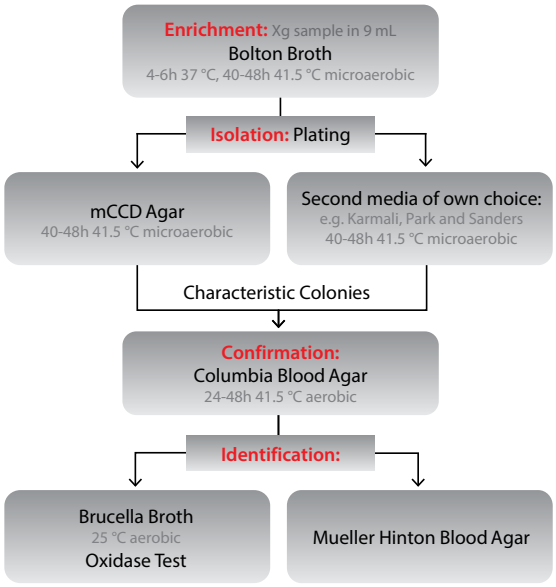

Figure 3: Flow diagram of ISO 10272-1: Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method

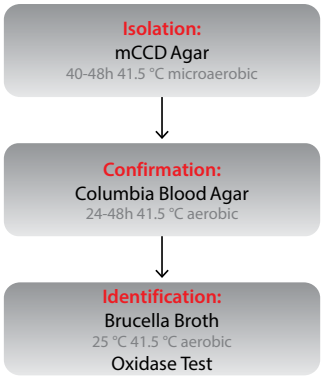

Figure 4: Flow diagram of ISO 10272-2: Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 2: Colony-count technique

supplement and a second selective medium. Suspect colonies are then confirmed on the Columbia Agar and then identification is usually done by colony morphology, biochemical testing, antibiotic susceptibility testing, immunological methods or by speciation through PCR-based methods.

"In situ" sandwich hybridization method for rapid detection

A further rapid and simple option to screen *Campylobacter* is the detection of specific rRNA. With oligonucleotide probes, specific sequences on the rRNA are detected and made visible with a sandwich hybridization followed by a chromogenic reaction. It is a very easy method and does not need any PCR, is not sensitive to sample matrix and detects only living cells, as rRNA is destroyed within a few hours. No special expensive equipment is needed and the test is done within approximately 2 to 2.5 hours. The test format is based on a 96-well microplate with 12 strips of 8 wells so that small numbers of samples can be screened. A positive result is visible to the naked eye; however, it is also possible by a standard microplate reader to quantify the number of cells at 450 nm. With a pre-enrichment step (44–48 hours in Bolton Broth), a sensitivity of 1 CFU/25 g can be reached. The HybriScan™*D Campylobacter* Kit specifically detects the most relevant *Campylobacter* spp. (*C. jejuni, C. coli, C. lari, C. upsaliensis*). HybriScan*D Campylobacter* was compared and validated (acc. to EN ISO 16140:2003) with the cultivation based method according to § 64 LFGB (official method in Germany). Five different food categories were tested. The results of the validation led to a relative accuracy of 95.2%, a relative specificity of 97.5%, and relative sensitivity of 93%, respectively.

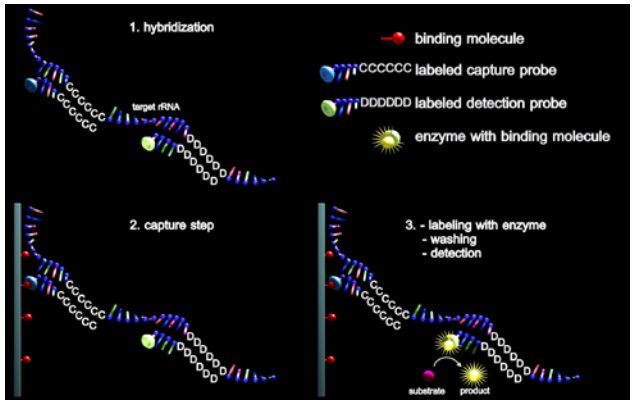

Figure 5: Principle of the HybriScan kit

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The Genus *Vibrio*

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Vibrio is a genus with many species, some of which cause waterborne diseases, while others are known symbionts for other marine organisms.

Vibrio is often found in open water, freshwater and saltwater. Some important pathogens are *Vibrio cholera*, which causes cholera in humans, and *Vibrio parahaemolyticus* and *Vibrio vulnificus*, which are the leading cause of seafood-associated gastroenteritis.

Vibrios are motile, curved or comma-shaped bacilli and have a single polar flagella with sheath proteins. The name *Vibrio* comes from Latin, and it is given this name because it possesses a flagellum and, under the microscope, it appears to vibrate. Vibrios are facultative aerobe and Gram-negative bacterium and do not form spores. The metabolism can be oxidative and fermentative. Most species are oxidase-positive except *V. metschnikovii*. Some vibrios like *Vibrio fischeri* are symbionts of fish, jellyfish, squid and other organisms. Through the quorum sensing mechanisms, (presence of the correct n-acyl homoserine lactones), they show bioluminescence. Based on this mechanism of communication, vibrios also build biofilms, regulate their virulence factors and other metabolic activities. In most ways, vibrios are close to Enterobacteriaceae, but also share some properties with pseudomonads. They can be differentiated from enteric bacteria by oxidase-positive reaction and motility. Differentiation from *Pseudomonas* can be made based on the ability of vibrios to undergo oxidative and fermentative metabolism.

Most vibrios are not fastidious and a simple C-source like glucose serves as an energy source. As it is typically a marine organism, for optimal growth most species need 2–4% NaCl or other salts and trace elements present in seawater. Some species are like *Pseudomonas* and can use diverse energy sources and show great versatility in their metabolism.

Did you know…

what "cholera" means?

Cholera comes from the Greek word meaning "bile" and is characterized by diarrhea and vomiting with bile expulsion.

Figure 1: *Vibrio cholera*

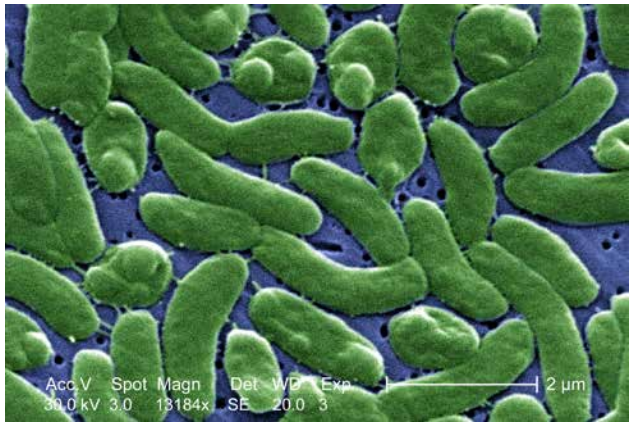

Figure 2: This scanning electron micrograph (SEM) depicts a grouping of *Vibrio vulnificus* bacteria. It normally lives in warm seawater and is part of a group of vibrios that are called "halophilic" because they require salt. *V. vulnificus* can cause disease in those who eat contaminated seafood or have an open wound that is exposed to contaminated seawater. Among healthy people, ingestion of *V. vulnificus* can cause vomiting, diarrhea, and abdominal pain. In immunocompromised persons, particularly those with chronic liver disease, *V. vulnificus* can infect the bloodstream, causing a severe and life-threatening illness characterized by fever and chills, decreased blood pressure (septic shock), and blistering skin lesions. *V. vulnificus* bloodstream infections are fatal about 50% of the time. (Source: CDC/ Colorized by James Gathany, 2005)

The widely used media for *Vibrio* isolation are TCBS Agar and Alkaline Saline Peptone Water. However, accompanying sucrose-fermenting bacteria may pose a problem in the identification of *Vibrio* species on TCBS Agar.[3] The TCBS Agar contains a mixed indicator bromothymol blue, and the thymol system reacts on acid production from sucrose fermentation. On a chromogenic medium like HiCrome™ *Vibrio* Agar (see **Table 2**), the color development by *Vibrio* species is not affected by the presence of colonies of other bacteria. This is because the amount of color developed depends on the reaction of the bacterial β-galactosidase with the substrate contained in the media. On the TCBS Agar, another indicator system is used, with sodium thiosulfate and ferric citrate, so the production of hydrogen sulphide is detected.

Pepton from animal origin provides carbonaceous, nitrogenous and essential nutrients to the *Vibrio* species to promote their growth. A high concentration of sodium chloride in the medium is used to get an inhibitory effect on the accompanying microflora. Sodium thiosulphate, sodium citrate and sodium cholate are also used to inhibit the growth of Gram-positive and some Gram-negative bacteria, but not members of Enterobacteriaceae. The strongly alkaline pH of the medium is also an important tool to get selectivity for *Vibrio* species.

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Vibrionales
Family: Vibrionaceae
Genus: *Vibrio*

	V. cholerae	*V. parahae-molyticus*	*V. vulnificus*
Growth in nutrient broth without NaCl	+	–	–
Growth in nutrient broth with 1% NaCl	+	+	+
Oxidase	+	+	+
Nitrate reduction	+	+	+
myo-Inositol fermentation	–	–	–
Arginine dihydrolase	–	–	–
Lysine decarboxylase	+	+	+
Ornithine decarboxylase	+	+	V
ONPG	+	+	+

Table 1: Typical biochemical reactions

	Name	Brand	Cat. No.
Nonselective media	Peptone Water	Fluka	**77185**
	Thiol Broth	Fluka	**T2117**
Nonselective media with differential system	OF Test Nutrient Agar	Fluka	**75315**
	Tryptic Soy Agar with supplement: TTC solution	Fluka	**22091**
	Glucose Salt Teepol Broth	Fluka	**49281**
Selective media	CPC-Agar (Base)	Fluka	**17134**
Selective media with differential system	DCLS Agar	Fluka	**70135**
	DCLS Agar No. 2	Fluka	**90035**
	HiCrome™ Vibrio Agar	Fluka	**92323**
	TCBS Agar (ISO 21872-1&2)	Fluka	**86348**

Table 2: Media for enrichment, detection and differentiation of *Vibrio* species

Figure 3: HiCrome Vibrio Agar (Fluka 92323), *V. cholera* as purple colonies

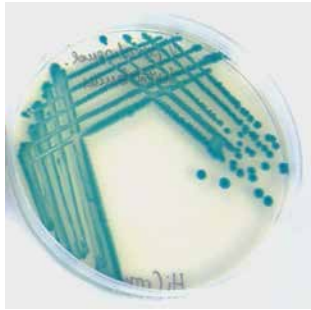

Figure 4: HiCrome Vibrio Agar (Fluka 92323), *V. parahaemolyticus* as bluish green colonies

V. cholerae is a non-invasive bacterium, affecting the small intestine by producing the cholera enterotoxins. The result is a life-threatening watery diarrhea because of activation of the adenylate cyclase in the intestinal cells. This reaction causes water and electrolytes from blood and tissues to be pumped into the intestinal tract. The rapid loss of fluid leads to dehydration, anuria, acidosis and shock. An additional loss of potassium ions may result in cardiac complications and circulatory failure. The mortality rate is very high (50–60%) if the disease is not treated. Infection source is water or food contaminated with human feces.

V. parahaemolyticus causes gastroenteritis. It is an invasive organism primarily affecting colon tissue commonly associated with the production of two toxins, thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH). But there was also other clinical samples found where other toxins like for example the thermolabil hemolysin (TL).[7,8] In most cases, the origin of an infection leads back to contaminated, raw and improperly refrigerated seafood or fecal contamination of water and food.

V. vulnificus lives in warm seawater and is halophilic, meaning it requires salt for growth. Contaminated seafood which is eaten raw or is undercooked is in most cases the source of infection and the cause of gastroenteritis, or a syndrome known as "primary septicemia." Also, open wounds that are exposed to seawater can lead to a wound infection.

Name	Brand	Cat. No.
Polymyxin B Selective Supplement	Fluka	**P9602**
TTC Solution	Fluka	**17779**

Table 3: Supplements for *Vibrio* media

Name	Brand	Cat. No.
Aminopeptidase Test (detection of Gram-negative microrganisms)	Fluka	**75554**
Oxidase Reagent acc. Gordon-McLeod	Fluka	**18502**
Oxidase Strips	Fluka	**40560**
Oxidase Test	Fluka	**70439**
Oxidase Reagent acc. Gaby-Hadley A	Fluka	**07345**
Oxidase Reagent acc. Gaby-Hadley B	Fluka	**07817**
ONPG Disks	Fluka	**49940**
Nitrate Reagent Disks Kit	Fluka	**51138**
Nitrate Reagent A	Fluka	**38497**
Nitrate Reagent B	Fluka	**39441**
Gram Staining Kit	Fluka	**77730**

Table 4: Biochemical products and kits for *Vibrio* identification and differentiation

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Fungi Identification

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Fungi are heterotrophic organisms that were previously included in the Kingdom Plantae, and today are classified as a separate kingdom, MYCOTA.

The Kingdom Fungi comprises unicellular organisms (e.g., yeasts), molds (e.g., fungi of the genera *Aspergillus, Penicillium, Fusarium*) and the Basidiomycetes, including the well-known edible and poisonous cap mushrooms. Molds are composed of filamentous hyphae that form an interconnected network known as mycelium, on which species-specific spores of the imperfect stage (most often asexual conidiospores) are produced, sometimes accompanied by sexual spores. Their typical size is from several to several dozen micrometers, so they are referred to as "microscopic fungi", since their structures can only be seen under the microscope. Today, microscopy and media are still the most used tools to identify fungi, although it can also be done with PCR. The process for the classical steps in the identification of fungi:

1. Homogenization / Mixing of the sample:
2. Decimal dilution of the homogenate
3. Plating of the dilution on the corresponding media or inoculation into a liquid broth
4. Isolation, purification and identification of the colonies

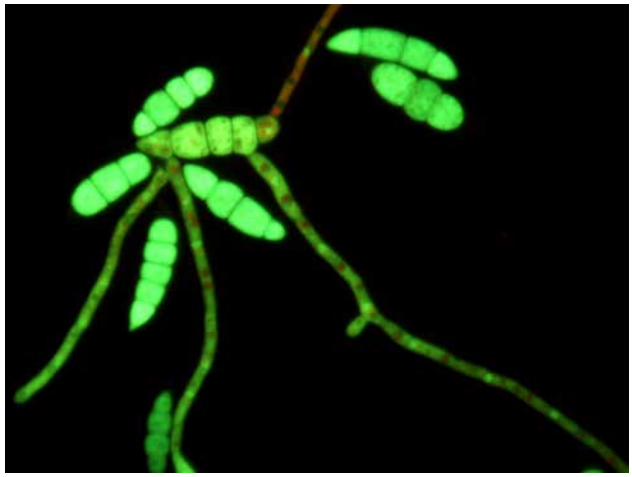

Figure 1: *Fusarium culmorum* conidia (Packa Danuta, University of Warmia Mazury)

The media are used for the enrichment of fungi, to perform a count and to get single pure colonies which can be isolated and used for further identification. In the identification, the media can be used for biochemical reactions or selective growth, but mainly they are used for the morphology and to get the typical form and spores for examination under the microscope.

Cat. No.	Medium	Enrichment	Isolation / Enumeration	Detection	Identification	Media System
73608	BiGGY Agar		x	x	x	selective + differential system
40545	$CaCO_3$ Agar		x	x	x	nonselective + differential system
94382	Candida Ident Agar, modified		x	x	x	selective + differential system
42347	Corn Meal Agar		x	x	x	nonselective
70185	Czapek Dox Agar		x	x	x	nonselective
C1551	Czapek-Dox Broth	x				nonselective
40587	Dichloran Glycerol Agar		x	x		selective media
17147	Dichloran Rose Bengal Agar (Base)		x	x		selective media
D2560	DNase Test Agar with Toluidine Blue		x	x	x	nonselective + differential system
66481	HiCrome™ OGYE Agar Base*		x	x	x	selective + differential system
M6907	Malt Extract Agar		x	x		selective by low pH
70145	Malt Extract Agar		x	x		selective by low pH
38954	Malt Extract Agar modified, Vegitone		x	x		selective by low pH
97218	Malt Extract Agar, modified		x	x		selective by low pH
M6409	Malt Extract Broth	x				selective by low pH
70146	Malt Extract Broth	x				nonselective
O3506	Oatmeal Agar		x			nonselective
75310	OGY Agar		x	x	x	selective media
75405	Orange-serum Agar		x			selective by low pH
77196	Peptone Yeast Extract Agar		x			selective media
79883	Peptone Yeast Extract Agar		x			selective media

Table 1: Most important media for fungi
* Not sold in U.S.A.

Continued on following page

Cat. No.	Medium	Enrichment	Isolation / Enumeration	Detection	Identification	Media System
P2182	Potato Dextrose Agar		x			nonselective
P6685	Potato Dextrose Broth	x				selective by low pH
70139	Potato Glucose Agar		x			nonselective
51684	Potato Glucose Agar		x			nonselective
17204	Potato Glucose Rose Bengal Agar (Base)		x	x		selective media
R1273	Rose Bengal Agar Base		x	x		selective media
17211	Rose Bengal Chloramphenicol Agar		x	x		selective media
84086	Sabouraud 2% Glucose Agar		x			selective media
55277	Sabouraud 4% Glucose Agar		x			selective media
84088	Sabouraud 4% Glucose Agar		x			selective media
89579	Sabouraud Glucose Agar with Chloramphenicol		x	x	x	selective media
S3306	Sabouraud Glucose Broth	x				selective by low pH
84886	Selective Agar for pathogenic fungi		x	x		selective media
85463	Simmons Citrate Agar				x	nonselective + differential system
17222	WL Nutrient Agar		x	x		nonselective + differential system
70196	Wort Agar*		x			selective by low pH
53493	Wort Broth	x				selective by low pH
Y3127	Yeast Malt Agar		x			nonselective
Y3752	Yeast Malt Broth	x				nonselective
51483	Yeast Nitrogen Base				x	selective by low pH
95765	YGC Agar		x	x		selective media

Table 1: Most important media for fungi
* Not sold in U.S.A.

For the identification of fungi under the microscope, the shape of the mycelia and cells are of interest, but the more important identification properties are the shape, method of production and arrangement of spores (conidial ontogeny). As a contrast agent, dyes are chosen which stain typical fungal elements (mostly the cell wall) like chitin, cellulose or mucin, respectively the carbohydrates of this glycoprotein mixture. Below are listed some classical stains, and with Calcofluor White Stain, fluorescence microscopy is also possible. Another sensitive and specific fluorescence staining could be achieved with Atto-lectins which are excellent stains for fungi mycelia (more information on **sigma-aldrich.com** or in the Microbiology Focus Vol. 1.2 2009).

Staining Solutions for Fungi	Description	Cat. No.
Calcofluor White Stain	A fluorescent stain for rapid detection of yeasts, fungi and parasitic organisms. Calcofluor White is a non-specific fluorochrome that binds to cellulose and chitin in cell walls.	18909
Lactophenol Blue Solution	Lactophenol Blue Solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. Fungal elements (chitin in the cell wall) are stained intensely blue.	61335
Mayer's Mucicarmine Stain Solution	Most mucins contain several glycoproteins and can therefore be detected by stains for carbohydrates. Best's carmine stain is specifically used for glycogens. Mucicarmine is an empirical stain. When applied for fungal staining, it stains the spores and hyphae from pale to dark red.	41325

Table 2: The most common stains for fungi and their spores

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