Microbiology Focus

Volume 7.3, 2015

Fluka® Analytical

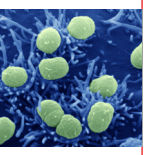
Escherichia coli the Best Known Bacteria

Escherichia coli is found in animal and human gut flora. Ground beef and unpasteurized milk are examples of food which can be sources of contamination. The scanning electron microscopy image shows a diarrheagenic *E. coli* strain inducing short pedestal-like formations, known as "attaching and effacing lesion", on HEp-2 cell surface. (Source: Abe Cecili, Instituto Butantan, Sao Paulo)

E. coli 2

Shigella 5

Fluorescence Staining 8



Escherichia coli

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A fecal indicator

E. coli is a Gram-negative, facultative anaerobic rod-shaped and motile bacterium belonging to the Enterobacteriaceae family. Its flagellar arrangement is peritrichous and highly fimbriated. The natural habitat is the intestine of humans and animals. Based on this, it is seen as a typical fecal indicator and their presence lets us assume that other pathogen organisms of fecal origin can be present. *Escherichia coli* got its name from the German pediatrician Theodor Escherich who first discovered it and coli from the Latin word colon, a part of the large intestine.

Even though they are normally present in our intestinal tract, *E. coli* themselves can be the cause of severe diseases, especially the enterovirulent *E. coli* such as the most famous *E. coli* O157. *E. coli* O157 belongs to the group of enterohemorrhagic *E. coli* (EHEC) strains and 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 EHECs the ability to adhere to intestinal epithelial cells.[2] In some cases, a heat stable toxin (astA gene) has been found on the bacteria chromosomes.[4] 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 p0157, where additional genes for the pathogenic mechanism are likely to be present as well.[3]

Did you know…

Some *E. coli* strains are probiotics?

Strains like *Escherichia coli* Nissle 1917 are known to be beneficial to the human body. They are approved for use in the treatment and prevention of gastrointestinal disorders.

Figure 1: *E. coli* bacteria.

E. coli possess the ability to transfer DNA by bacterial conjugation, transfection by bacteriophages and transformation, which is a problem since if an EHEC is in the intestine, it can turn other harmless *E. coli* into pathogens.

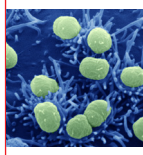
Water testing is one important topic with regard to *E. coli* as water is very important for production of medicine, food, beverages, and many other materials, and is also used to clean equipment, etc. The risk of fecal contamination of water is also very high depending on the source and treatment of water. Even in highly developed parts of the world or in villages close to a mountain with pristine water, there are still occasional cases of contamination.

- **Class** – Gammaproteobacteria
- **Order** – Enterobacteriales
- **Family** – Enterobacteriaceae
- **Genus** – *Escherichia*
- **Species** – *E. coli*

All *E. coli* can ferment lactose and build acid and gas during the fermentation process, which is the typical definition of all coliforms. In addition, under anaerobic conditions, they can ferment diverse carbohydrates, producing lactate, succinate, ethanol, acetate, and carbon dioxide as end products which can be detected by decreasing pH, or by gas production (see **Table 1**).

	Disc		Reaction	
Carbohydrates	Cat. No.	Brand	Acid	Gas
Adonitol	55876	Fluka®	–	–
Arabinose	80372	Fluka	+	+
Cellobiose	56481	Fluka	–	–
Dextrose	63367	Fluka	+	+
Dulcitol	73044	Fluka	–	–
Fructose	53901	Fluka	+	+
Galactose	89608	Fluka	+	+
Inositol	89614	Fluka	–	–
Lactose	28816	Fluka	+	+
Maltose	77653	Fluka	+	+
Mannitol	94438	Fluka	+	+
Mannose	94445	Fluka	+	+
Melibiose	93196	Fluka	+	+
Raffinose	94226	Fluka	–	–
Rhamnose	93999	Fluka	+	+
Salicin	92971	Fluka	–	–
Sorbitol	93998	Fluka	+	+
Sucrose	94309	Fluka	–	–
Trehalose	92961	Fluka	+	+
Xylose	07411	Fluka	+	+

Table 1: Carbohydrate discs and the typical reactions of *E. coli* such as ATCC 25922.

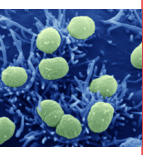


Cat. No.	Reagents and Tests	Description
29333	Barritt's Reagent A	These reagents are used in the Voges-Proskauer test for detection of acetoin production by bacterial culture
39442	Barritt's Reagent B	
49825	DMACA Reagent	For the detection from tryptophanase activity of organisms by verification of indole, which accrued from tryptophan decomposition
05686	DMACA Indole Discs	
67309	Kovac's Reagent	In the presence of oxygen, some bacteria, like *E. coli*, are able to split tryptophan into indole and alpha-aminopropionic acid. This reagent is for detecting the indole and identifying the indole-positive microorganisms
60983		
78719	Kovac's Reagent Strips	
08714	Methyl Red Solution	Some bacteria utilize glucose to form large amounts of acid with the result that the pH value of the medium falls distinctly. Other species produce no or less free acid. This difference can be visualized by using methyl red
38497	Nitrate Reagent A	The alpha-Naphtylamine and Sulphanilic solution is used to detect nitrate reduction by bacteria. Organisms with nitrate reductase reduce nitrate to nitrite which reacts with sulphanilic acid to form a diazonium salt. With alpha-naphtylamine, the salt is converted into a red azo dye
39441	Nitrate Reagent B	
73426	Nitrate Reduction Test	Bacterial species may be differentiated on the basis of their ability to reduce nitrate to nitrite or nitrogenous gases. The reduction of nitrate may be coupled to anaerobic respiration in some species
07689	O'Meara's Reagent	The reagent is used in the Voges-Proskauer test for the detection of acetoin production by bacterial cultures

Table 2: Reagents and tests for identification and confirmation of *E. coli*.

Media System	Cat. No.	Name
Non-selective differential media	39484	Methyl Red Voges Proskauer Broth
	72548	Nitrate Broth
	75315	OF Test Nutrient Agar
	85438	SIM Medium*
	44940	Triple Sugar Iron Agar
	92499	Triple Sugar Iron Agar (acc. to ISO)*
Non-selective media	M1053	Motility Test Medium
	93657	Tryptone Medium
	70194	Tryptone Water
	07507	Tryptone Water*
	39964	Tryptone Water, Vegitone
	09136	Tryptophan Broth
Selective differential media	31432	DEV ENDO Agar*
	70186	EMB Agar
	E5399	Endo Agar
	70137	ENDO Agar (Base)
	48716	Gassner Agar
	54232	Lactose TTC Agar with Tergitol®-7
	62087	Levine EMB Agar
	70143	MacConkey Agar No. 1
	M8302	MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15% Bile Salts
	94216	MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15% Bile Salts* (ready to use media in flask)
	70144	MacConkey Broth
	16377	MacConkey Broth Purple
	63014	MacConkey MUG Agar
	51405	MacConkey-Agar (without salt)
	17171	Mineral modified Glutamate Broth (Base)
	96961	m-FC Agar
	43291	m-FC Agar, Vegitone
	17184	m-HD Endo Broth with Brilliant Green
	86455	Tergitol-7 Agar
	70188	Violet Red Bile Agar
	42376	Violet Red Bile Agar, Vegitone
	70189	Violet Red Bile Glucose Agar*
	17213	Violet Red Bile Glucose Agar without Lactose
	53605	Violet Red Bile Glucose Agar without Lactose, Vegitone
Selective media	17112	A1 Broth
	16025	Brilliant Green Bile Lactose Broth
	92008	Tryptone Bile Agar

Table 3: An excerpt from the Sigma-Aldrich® range of typical media to enrich, identify and enumerate *E. coli* (more media can be found on **sigma-aldrich.com/microbiology**).
* Not sold in USA.



Also a broad range of other substrates can be used by *E coli* triggered by aerobic or anaerobic respiration such as organic acids, amino acids, nitrates, and many more. Some interesting biochemical confirmation tests to confirm or differentiate *E. coli* are the indole test where the tryptophanase activity of *E. coli* is detected, the positive methyl red Voges Proskauer test and the positive nitrate test. The organism is not able to use citrate as an energy source and can't hydrolyze urea and gelatin. Some typical examples of tests and reagents are listed under **Table 2**. There is also a wide range of media available for *E. coli* and there are a lot of non-selective and selective media often combined with a biochemical differential system (see an excerpt of the Sigma-Aldrich range in **Table 3**) and also those specifically used for the enteropathogenic *E. coli* (see **Table 4**).

ISO 16649-1:2001

Horizontal method for the enumeration of β-glucuronidase-positive *E. coli*

Part 1: Colony-count technique at 44 °C using membranes and 5-bromo-4-chloro-3-indolyl β-D-glucuronide

ISO 16649-2:2001

Horizontal method for the enumeration of β -glucuronidase-positive *E. coli*

Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide

ISO 16649-3:2015

Horizontal method for the enumeration of β-glucuronidase-positive *E. coli*

Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl- β-D-glucuronide

ISO 7251:2005

Horizontal method for the enumeration of presumptive *E. coli*

Most probable number technique

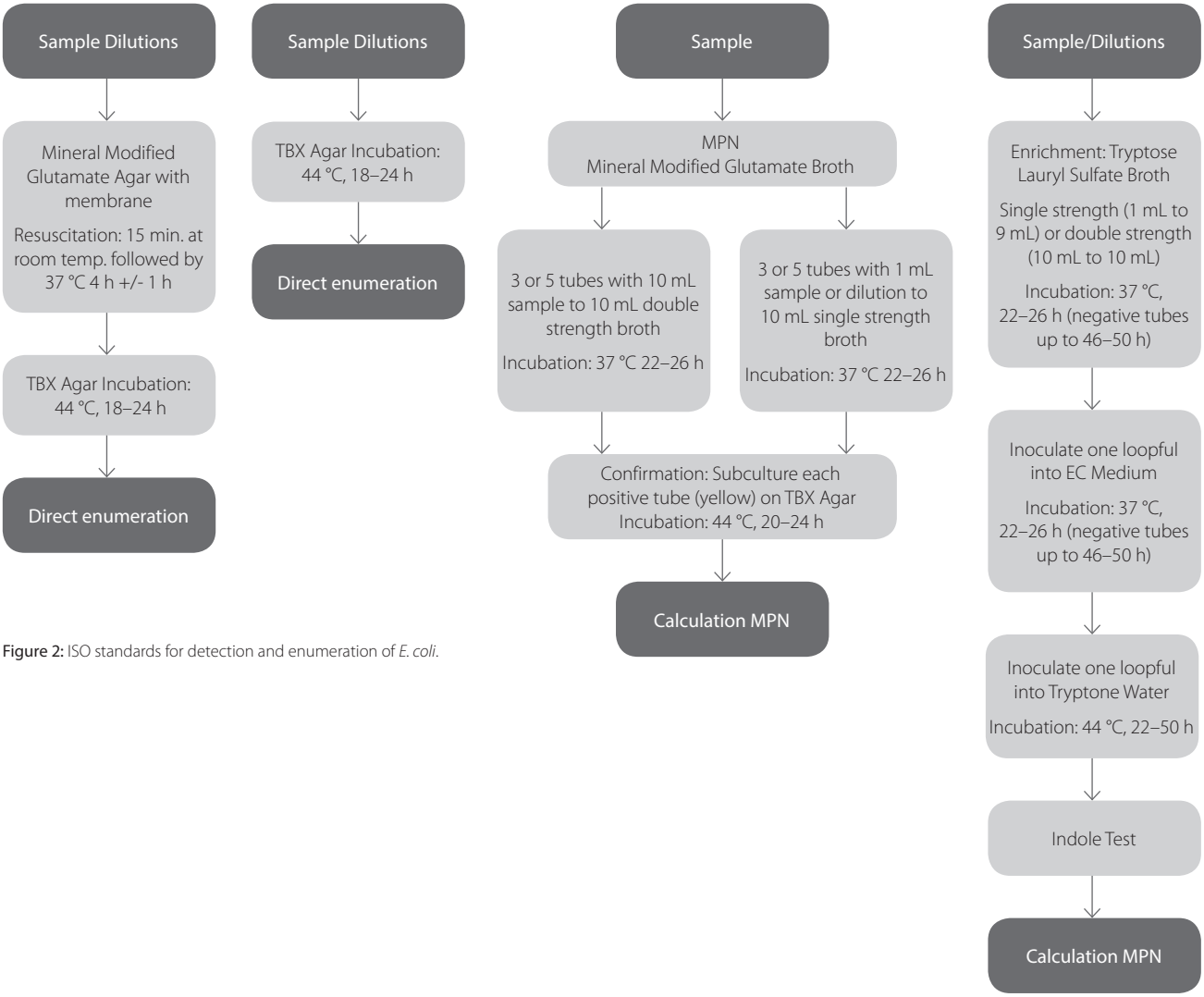
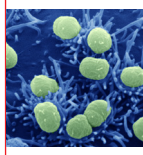


Figure 2: ISO standards for detection and enumeration of *E. coli*.



Currently, interesting methods are used for the detection and differentiation of *E. coli* by chromogenic or fluorescence substrates, the most popular ones being the substrates used to detect the β-glucuronidase (e.g. methylumbelliferyl-β-D-glucuronide = MUG, 5-bromo-4-chloro-3-indolyl β-D-glucuronide = X-glu, but other enzymes like glucosidase and galactosidase are also used. Actually Sigma-Aldrich provides a huge range of such innovative media. Have a look at the complete list in **Table 5**.

Most *E. coli* strains are thermotolerant, and some strains even grow at 49 °C,[9] but optimal growth is at 37 °C. *E. coli* 0157:H7 demonstrates no or limited growth at 44–45.5 °C, particularly in the presence of 0.15% bile salts.[6] Sometimes elevated temperatures for incubation are used as an additional selectivity for *E. coli*. The elevated temperature is also used in the ISO standard protocols to detect and enumerate *E. coli*, (see flow process in **Figure 2**).

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

Table 4: List of media for detection of enteropathogenic *E. coli*.
* Not sold in USA.

Media System	Cat. No.	Name
Non-selective chromogenic medium	73009	HiCrome ECC Agar
Selective chromogenic media	81938	HiCrome Coliform Agar
	70722	HiCrome *E. coli* Agar B
	85927	HiCrome ECC Selective Agar
	09142	HiCrome ECD Agar with MUG
	90924	HiCrome m-TEC Agar
	51489	HiCrome Rapid Coliform Broth
	39734	Membrane Lactose Glucuronide Agar
	92435	TBX Agar
Non-selective fluorescence media	17165	MUG Tryptone Soya Agar
	51413	Plate Count MUG Agar
Selective fluorescence media	16016	BRILA MUG Broth*
	44657	ECD MUG Agar*
	62634	LST-MUG Broth
	M1678	MUG EC Broth
	95273	VRB MUG Agar
	44655	ECD Agar*

Table 5: List of chromogenic and fluorogenic media for detection of *E. coli*.
* Not sold in USA.

Reference

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2. M. Vlidal, et al., "Distribution of putative adhesins in Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from different sources in Chile," Epidemiol Infect., 135(4): 688–694 (2007).
3. P. Boerlin, et al., "Associations between Virulence Factors of Shiga Toxin-Producing *Escherichia coli* and Disease in Humans," J Clin Microbiol., 37(3): 497–503 (1999).
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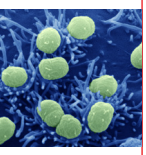

Shigella

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A pathogen which can cause bloody diarrhea and in 15% of the cases, ends in death.[10]

Bacteria of the genus *Shigella* are Gram-negative, facultative anaerobic, non-motile rods. *Shigella* was first discovered in 1896 by the Japanese microbiologist, Kiyoshi Shiga. It was initially called *Bacillus dysenteries*, and the exotoxin from *S. dysenteriae*, called Shiga toxins (also called verotoxins), got its name from the discoverer.[3] It is related to the Shiga-like toxins which are produced from the enterohemorrhagic *E. coli* (EHEC) like *E. coli* O157. Additionally, *Shigella* can produce *Shigella* enterotoxin 1 and 2[3] and also a protease called VirA, which destabilizes the microtubules in human cells, allowing the bacteria to enter the cell[4,5] and for the actin-mediated motility.[6,7] It is very resistant to acid in the human stomach and also to proteases present in the intestinal tract. That is also the reason that even small numbers can lead to an outbreak of shigellosis. Of the various strains, *S. sonnei* is the cause of 77% of infections in the developed world (summer diarrhea). *S. flexneri* is the most reported *Shigella* worldwide (60% in developing countries) and *S. boydii* are responsible for most of the remaining cases while *S. dysenteriae* is very seldom seen in developed countries but accounts for 30% of cases in underdeveloped areas.[8]

- **Class** – Gammaproteobacteria
- **Order** – Enterobacteriales
- **Family** – Enterobacteriaceae
- **Genus** – *Shigella*

Shigella bacteria are the cause of shigellosis which occurs primarily in humans. It is frequently found in water polluted with human feces and the transmission is mainly from person to person due to poor hygiene, polluted water, food or sometimes flies. The typical incubation time is 12 to 96 hours and recovery takes 5 to 7 days.[1] The symptoms range from mild abdominal discomfort to bloody diarrhea, fever, and stomach cramps.[2]

Did you know…

Shigella is genetically closely related to _E. coli_?

Both genera belong to the tribes of Escherichieae, but *E. coli* is biochemically very active while Shigella just ferments a few carbohydrates.

Figure 1: This illustration is a computer-modified scanning electron micrographic image of drug-resistant *Shigella* bacteria. Note that the exterior of the *Shigella* bacterium is fimbriated.
Source: CDC, James Archer, 2013.

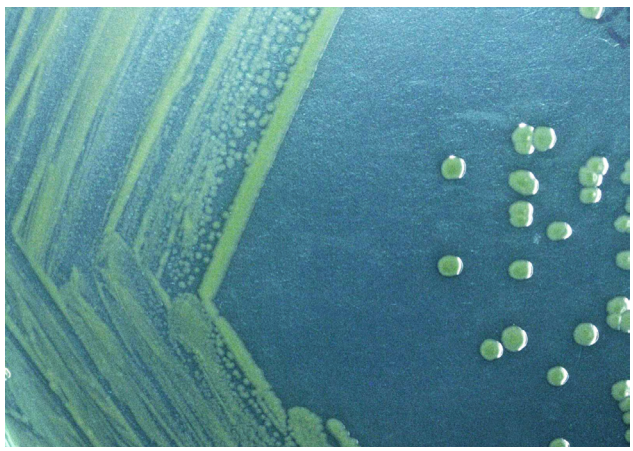


Figure 2: *Shigella sonnei* as bluish-green colonies on Hektoen enteric agar.
(Source: CDC/ Dr. Todd Parker. Ph.D, 2014)

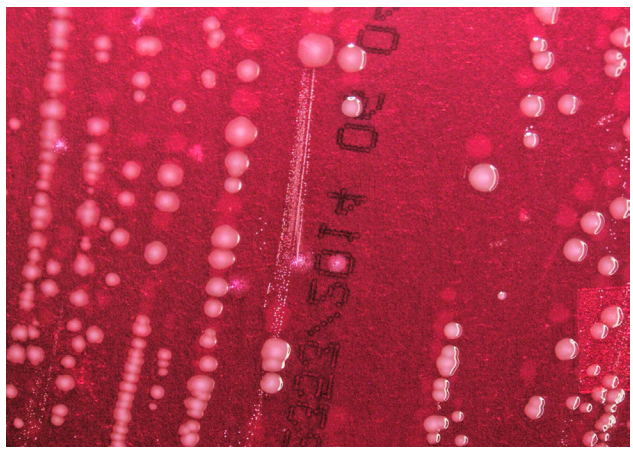
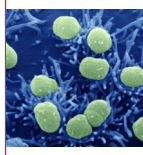
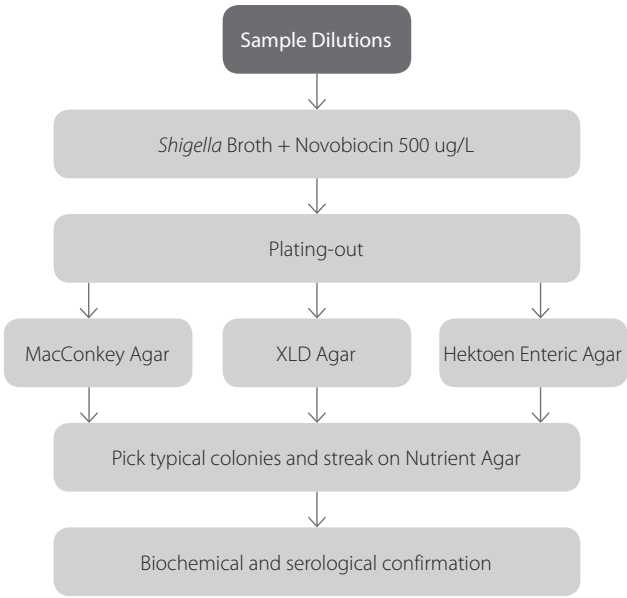


Figure 3: *Shigella sonnei* as reddish colonies on XLD agar.
(Source: CDC/ Dr. Todd Parker. Ph.D, 2014)



Shigella can ferment glucose and can reduce nitrates to nitrites without H_2S production. The end products of the anaerobic metabolism are acetic acid, lactic acid, succinic acid, formic acid and ethanol. Unlike the other species, *S. sonnei* can slowly ferment lactose.[9] *Shigella* typically do not produce gas from carbohydrates (with the exception of certain aerogenic strains of *S. flexneri*). They are methyl red positive as they produce large amounts of acids from glucose and are urea negative. Except for *S. dysenteriae*, all *Shigella* species can ferment mannitol. *S. sonnei* is indole negative, while the other species are variable. *S. sonnei* is also the only species from the genus which is ornithine and ONPG positive (process beta-galactosidase). On the Kligler Iron Agar or Triple Sugar Iron Agar, the slant surface shows alkaline reaction and on the butt shows the acidic reaction. Of course there is no H_2S production and no gas formation. On Hektoen enteric agar *Shigella* will appear as bluish-green colonies while *Salmonella* show bluish-green colonies with a black center. On the MacConkey Agar the colonies of *Shigella* stay colorless as they do not ferment lactose and on XLD agar the colonies are red as they are the only enteric bacteria which do not ferment xylose.



ISO 21567:2004 – Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Shigella spp*.

Cat. No.	Medium
70135	DCLS Agar
90035	DCLS Agar No. 2
D7809	Deoxycholate Citrate Agar
51490	Hektoen Enteric Agar
60787	Kligler Agar
61792	Leifson Agar
70143	MacConkey Agar No. 1
94216	MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15% Bile Salts (ready to use flasks)*
M8302	MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15% Bile Salts
72548	Nitrate Broth
75315	OF Test Nutrient Agar
85438	SIM Medium*
85640	SS-Agar
44940	Triple Sugar Iron Agar
92499	Triple Sugar Iron Agar (acc. to ISO)*
95586	XLD Agar
14781	XLD Agar ISO 6579:2002
39484	Methyl Red Voges Proskauer Broth

Table 1: Media for *Shigella* differentiation and identification.

References:

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Atto Dyes for Superior Fluorescent Imaging

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Activated fluorescent dyes are routinely used to tag proteins, nucleic acids, and other biomolecules for use in life science applications including fluorescence microscopy, flow cytometry, fluorescence *in situ* hybridization (FISH), receptor binding assays, and enzyme assays. The Atto dyes are a series of fluorescent dyes that meet the critical needs of modern fluorescent technologies:

- **Stability** – Atto 655 and Atto 647N, for example, are photostable and highly resistant to ozone degradation
- **Long Signal Lifetimes** – Signal decay times of 0.6–4.1 nanoseconds allow timegate studies to reduce autofluorescence background and scattering
- **Reduced Background** – Several Atto dyes employ excitation wavelengths greater than 600 nm, reducing background fluorescence from samples, Rayleigh and Raman scattering
- **Selection** – Atto dyes have strong fluorescent signals that cover visible and near-IR emission wavelengths

Atto Phalloidin

Phalloidin is a fungal toxin isolated from the poisonous mushroom *Amanita phalloides*. Its toxicity is attributed to the ability to bind F actin in liver and muscle cells. As a result of binding phalloidin, actin filaments become strongly stabilized. Phalloidin has been found to bind only to polymeric and oligomeric forms of actin, and not to monomeric actin. The dissociation constant of the actin-phalloidin complex has been determined to be on the order of 3×10^{-8}.

Phalloidin differs from amanitin in rapidity of action; at high dosage levels, death of mice or rats occurs within 1 or 2 hours. Fluorescent conjugates of phalloidin are used to label actin filaments for histological applications. Some structural features of phalloidin are required for the binding to actin. However, the side chain of amino acid 7 (g-ddihydroxyleucine) is accessible for chemical modifications without appreciable loss of affinity for actin.

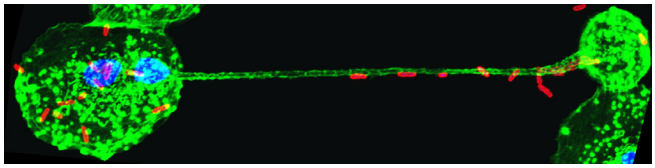

Figure 1: Confocal Laser Scanning Micrograph (CLSM) of human peripheral blood monocytes differentiated to macrophages, and infected with a red fluorescent *Serratia liquefaciens*. A macrophage tries to capture bacteria by means of a long pseudopod. Bacteria seem to walk along this structure. Fixed cells were permeabilized with Triton X-100. Atto-488 phalloidin (Sigma Aldrich) binds polymerized F-actin, 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. Image was kindly provided by Prof. Jose Ramos Vivas.

Name	Recommended λex / λem	Cat. No.
Phalloidin–Atto 390 conjugate	390 / 472 nm 0.1 M phosphate	50556-10NMOL
Phalloidin–Atto 425 conjugate	436 / 484 nm 0.1 M phosphate pH 7.0	66939-10NMOL
Phalloidin-Atto 430LS conjugate	433 / 520 nm 0.1phosphate pH 7.0	78999-10NMOL
Phalloidin-Atto 488 conjugate	501 / 523 nm 0.1 M phosphate pH 7.0	49409-10NMOL
Phalloidin-Atto 490LS conjugate	496 / 636 nm 0.1 M phosphate pH 7.0	14479-10NMOL
Phalloidin-Atto 520 conjugate	516 / 538 0.1 M phosphate pH 7.0	54367-10NMOL
Phalloidin- Atto 514 conjugate	511 / 533 0.1 M phosphate pH 7.0	94507-10NMOL
Phalloidin–Atto 532 conjugate	532 / 553 nm in 0.1 M phosphate pH 7.0	49429-10NMOL
Phalloidin–Atto 550 conjugate	554 / 574 nm in 0.1 M phosphate pH 7.0	19083-10NMOL
Phalloidin–Atto 565 conjugate	563 / 592 nm in 0.1 M phosphate pH 7.0	94072-10NMOL
Phalloidin–Atto 590 conjugate	594 / 624 nm in 0.1 M phosphate pH 7.0	93042-10NMOL
Phalloidin_Atto 594 conjugate	601/ 627 nm in 0.1 M phosphate pH 7.0	51927-10NMOL
Phalloidin–Atto 633 conjugate	629 / 651 nm in 0.1 M phosphate pH 7.0	68825-10NMOL
Phalloidin–Atto 647N conjugate	644 / 669 nm in 0.1 M phosphate pH 7.0	65906-10NMOL
Phalloidin–Atto 655 conjugate	663 / 684 nm in 0.1 M phosphate pH 7.0	18846-10NMOL
Phalloidin–Atto 665 conjugate	663 / 684 nm in 0.1 M phosphate pH 7.0	04497 -10NMOL
Phalloidin–Atto Rho6G conjugate	535 / 560 nm in 0.1 M phosphate pH 7.0	55212-10NMOL
Phalloidin-Atto 700 conjugate	700 / 719 nm in 0.1 M phosphate pH 7.0	79286-10NMOL
Phalloidin-Atto 725 conjugate	725 / 755 in 0.1 M phosphate pH 7.0	51865-10NMOL
Phalloidin-Atto 740 conjugate	750 / 764 in 0.1 M phosphate pH 7.0	07373-10NMOL

Table 1: List of Atto Phalloidin conjugates.

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