Microbiology Focus

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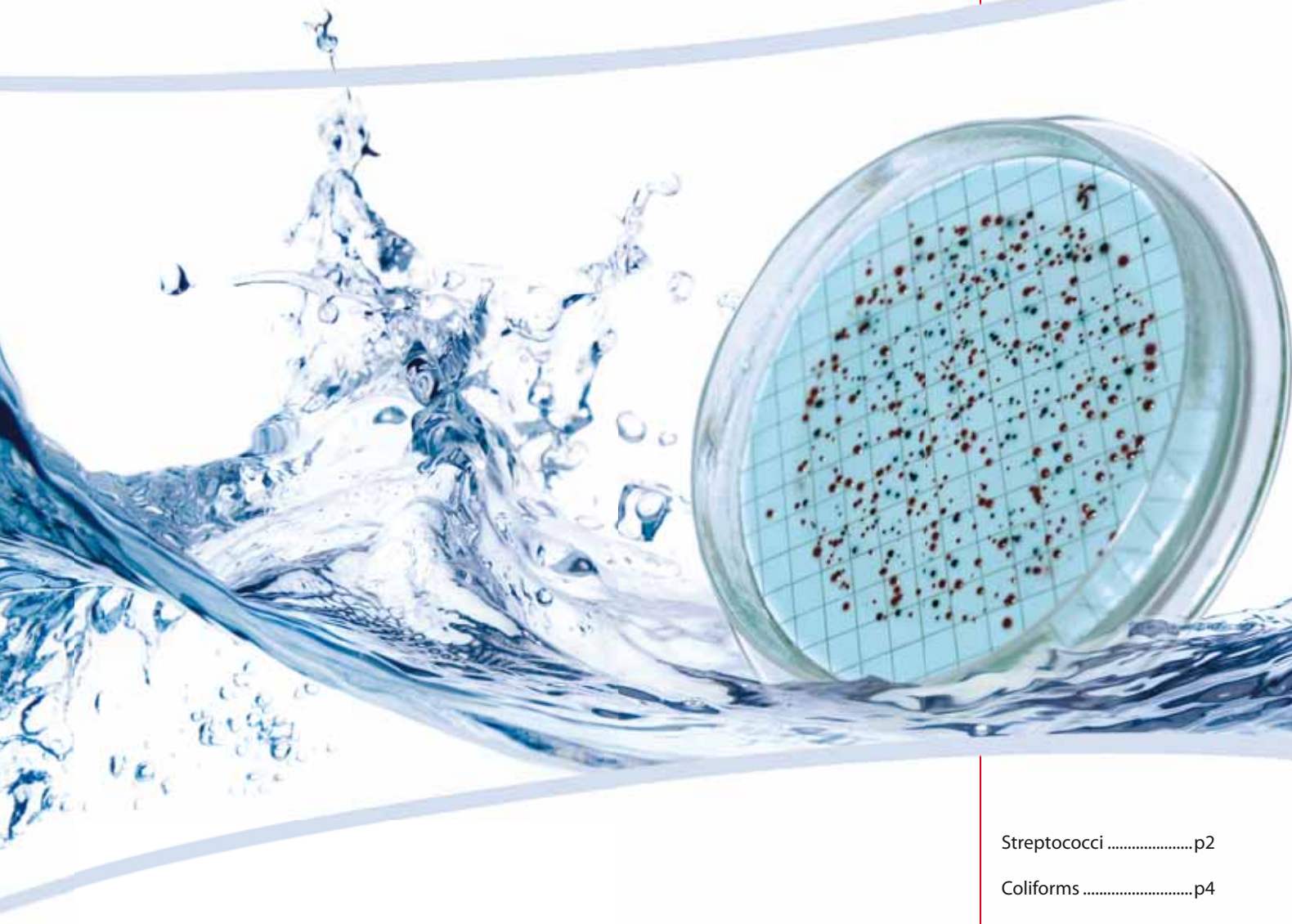
Fluka Analytical

Methods and Techniques for Detection of Coliforms



Coliforms from a water sample growing on a membrane filter on a differentiation media.

Streptococci

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

Detection, identification, differentiation and cultivation of streptococci

Streptococci are non-motile, microaerophilic, Gram-positive, spherical shaped bacteria (cocci). They often occur as chains or pairs and may be either facultative or strict (obligate) anaerobes. The catalase test is negative (while *Staphylococcus* is catalase-positive), and streptococci are not able to synthesize cytochromes (no oxidative phosphorylation). Streptococci are able to ferment sugars, however the end product is always lactic acid; therefore, they are very acid tolerant and are classified in the lactic acid bacteria group. The natural sources of streptococci are broad. They have been found to inhabit humans as well as a number of diverse animals, and are often found colonizing the mucosal surfaces of the mouth, intestinal tract, nares and pharynx. In drinking water, *Streptococcus* is an indicator of faecal contamination.

Food sources with high risk of contamination include milk and dairy products, eggs, steamed lobster, ground ham, potato salad, custard, rice pudding, and shrimp salad. In most cases, the food items have been exposed at room temperature for several hours between preparation and consumption. The contamination of the food is the result of poor hygiene, contact with infected humans, or the use of unpasteurized milk. However, there are also some Streptococci which are very welcome in the production of cheeses and yogurts, e.g. *Streptococcus lactis, Streptococcus cremoris, Streptococcus diacelillactis* and *Streptococcus thermophilus*, the most famous strain.

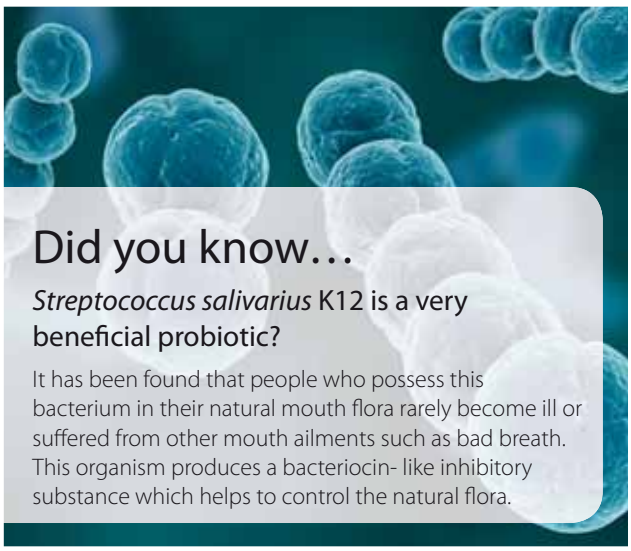


Figure 1: Streptococci

For detection, identification, differentiation, enumeration and cultivation of streptococci, Sigma-Aldrich provides a broad range of specific agars and broths (**Table 2**).To ensure accurate differentiation and identification, the Gram staining kit (Fluka 77730) and associated required tests (**Table 3**) are also available.

Streptococci are grouped by three types of hemolysis (α, β, γ). This reaction can be seen on sheep's blood agar plates (see Nonselective Agars for Differentiation in **Table 2**). This reaction is still a major test for biochemical characterization, followed by the catalase test.

- **α-Hemolytic Streptococci** - α-hemolysis indicates that the red blood cells are intact, but the hemoglobin is converted to biliverdin. This causes a greening of the blood agar plate around the colonies.
- **β-Hemolytic Streptococci** - β-hemolysis is a true hemolysis of erythrocyte by the enzyme hemolysin. Clear zones will appear around the colonies on the blood agar plate.
- **Non-Hemolytic or γ-Hemolytic Streptococci** - γ-hemolysis is a misnomer; there is actually no hemolysis.

The Lancefield antigens are diverse antigens isolated from streptococci and are often designated with the letters A, B, C, D, etc. This procedure is a good starting point for differentiation; however, it does not always help in identifying the organism.

Figure 2: Identification flow chart for gram-positive cocci

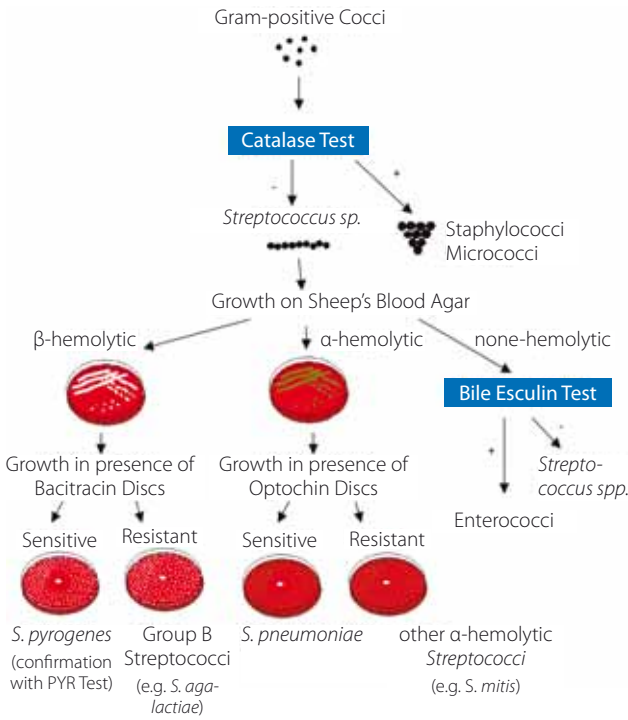


Table 1: Biochemical Characteristics of Streptococci and Enterococci (source: Koneman's Color Atlas and Textbook of Diagnostic Microbiology)

	Group A streptococci	Group B streptococci	Group C, F, G streptococci	Group D streptococci	Group D enterococci	Viridans streptococci	Pneumococci
typical species	*S. pyogenes*	*S. agalactiae*	*S. equi* *S. canis* *S. anginosus*	*S. bovis*, *S. suis*	*E. faecalis* *E. faciem* *E. durans* *E. avium*	*S. mutans* *S. salivarius*	*S. pneumoniae*
hemolysis	β	β, none	β	α, none	α, β, none	α, none	α
bacitracin	S	R	R	R	R	V	V
STX	R	R	S	S	R	S	S
CAMP Tests	-	+	-	-	-	-	-
hippurate	-	+	-	-	V	-	-
LAP	+	+	+	+	+	+	+
PYR	+	-	-	-	+	-	-
bile esculin	-	-	-	+	+	V	-
growth 6.5% NaCl	-	V	-	-	+	-	-
Optochin	R	R	R	R	R	R	S
bile solubility	-	-	-	-	-	-	+

STX = sulfamethoxazole-trimethoprim, LAP = leucine aminopeptidase, PYR = pyrrolidonyl peptidase, R = resistant, S = sensitive

Table 2: Fluka Brand Media for Streptococci

Selective Enrichment Broths	Cat. No.
Azide Glucose Broth*	11539
Glucose Azide Broth	17157
Nonselective Agars for Differentiation	**Cat. No.**
Blood Agar (Base)	70133
Blood Agar Base No. 2	B1676
CLED Agar	55420
Deoxyribonuclease Test Agar	70136
Deoxyribonuclease Test Agar	30787
DNase Test Agar with Toluidine Blue	D2560
LS Differential Agar	17153
Selective Agar for Detection and Isolation	**Cat. No.**
Streptococcus Selective Agar*	85874
Selective Agars with Differential System for Differentiation, Detection and Isolation	**Cat. No.**
Azide Blood Agar (Base)*	70132
Bile Esculin Azide Agar	06105
Kanamycin Esculin Azide Agar	17151
KF-Streptococcus Agar	60641
Mitis Salivarius Agar	01337
Medium for Sensitivity Testing	**Cat. No.**
Antibiotic Broth*	70184
Mueller-Hinton Agar 2	97580

* not available in USA

Table 3: Fluka Brand Tests for Identification and Differentiation of Streptococci

Test	Cat. No.
Bacitracin Disks *Identification of group A β-hemolytic streptococci from other β-hemolytic streptococci. (sensitive = presumptive group A streptococci)*	08382
Bile Esculin Disks *Detection of esculin hydrolysis in presence of bile for differentiation of group D streptococci from non-group D streptococci.*	80507
Hippurate Disks *Detection of hippurate hydrolase, differentiation of β-hemolytic group B streptococci*	40405
Optochin Disks *Differentiation of pneumococci and viridans streptococci. (Optochin inhibits pneumococci)*	74042
PYRase Strips (Pyrrolidonyl Peptidase Strips) *Differentiation of enterococci and Streptococcus pyogenes from other streptococci. (pyrrolidonyl peptidase = pyrase)*	67886

More details about the media and tests can be found on our website:
sigma-aldrich.com/microbiology

References:

1. M.J. Patterson, Streptococcus, In: Baron's Medical Microbiology (Baron S et al, eds.), 4th ed., Univ. of Texas Medical Branch (1996)
2. R. Facklam, What happened to the streptococci: overview of taxonomic and nomenclature changes, Clin. Microbiol. Rev. 15 (4), 613-30 (2002)
3. V. A. Getting, S. M. Wheeler, G. E. Foley, A Food-Borne Streptococcus Outbreak, Am. J. Public. Health Nations Health, 33(10), 1217–1223 (1943)

Methods and Techniques for Detection of Coliforms

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

Detection and enumeration of coliforms

By definition, coliforms are lactose-fermenting Enterobacteriaceae that produce acid and gas during the fermentation process of lactose. They are rod-shaped, Gram-negative, aerobic or facultative anaerobic bacteria. They are typically an indicator of faecal contamination and may co-occur with other pathogenic organisms of faecal origin. Although coliforms may be a normal part of our intestinal tract flora, they can be the cause of diverse infections, especially for elderly people or children.

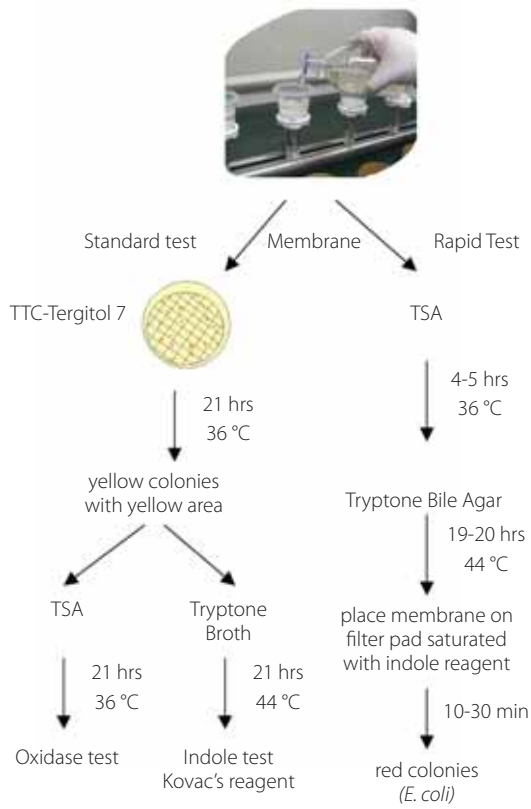
The following genera of bacteria are characteristically coliforms: *Citrobacter, Enterobacter, Escherichia* and *Klebsiella*.

Water testing is an important topic concerning coliforms. Water is vital in the production of may items, including pharmaceuticals, food, and beverages, and water is also used in the cleaning of production equipment. Depending on the source and treatment of the water used, the risk of faecal contamination can be very high. Even in highly developed regions of the world or in villages close to mountains with pristine water, cases of contamination still occur from time to time.

For the detection and enumeration of coliforms, ISO recommends a method utilizing the classical media and the biochemical reagents (see Figures 1-3). In addition, a variety of new chromogenic media is now available; the use of these media enables the analyst to replace some steps of the process and to realize advantages such as additional confirmation, faster results, less reagents, and reduced labour intensity. Table 1 lists some of the interesting chromogenic and fluorogenic media for *E. coli* and other coliforms. The methodology behind such media is a smart combination of selective agents and the differentiation by detection of characteristic enzymes with corresponding chromogenic and fluorogenic substrate.

In addition, Sigma-Aldrich also provides a comprehensive range of classical media to detect, identify and enumerate coliforms according to specific guidelines and regulations. In addition, we have expanded our product line to include some new interesting, specific and innovative media. A complete list of 200 different media can be found at ***sigma-aldrich.com/coliforms***

Figure 1: Detection and enumeration of *E. coli* and coliform bacteria in water acc. to EN-ISO 9308-1:2000



Oxidase neg.. indole pos. = *E. coli*

Oxidase neg.. indole neg. = Coliforms

Description	Cat. No.
Lactose TTC Agar with Tergitol®-7	54232-500G
Tryptic Soy Agar	79872-10X100ML*
	22091-500G
	22091-2.5KG
30 plates, ø55mm	57994-30EA-F*
Kovac's Reagent for indoles	67309-100ML
Oxidase Strips	40560-100STRIPS-F
Oxidase Test	70439-50DISKS-F
Oxidase Reagent acc. Gordon-McLeod	18502-100ML-F
Oxidase Reagent acc. Gaby-Hadley A	07345-100ML-F
Oxidase Reagent acc. Gaby-Hadley B	07817-100ML-F

* not sold in USA

Figure 2: Method for the enumeration of coliforms - MPN technique in food

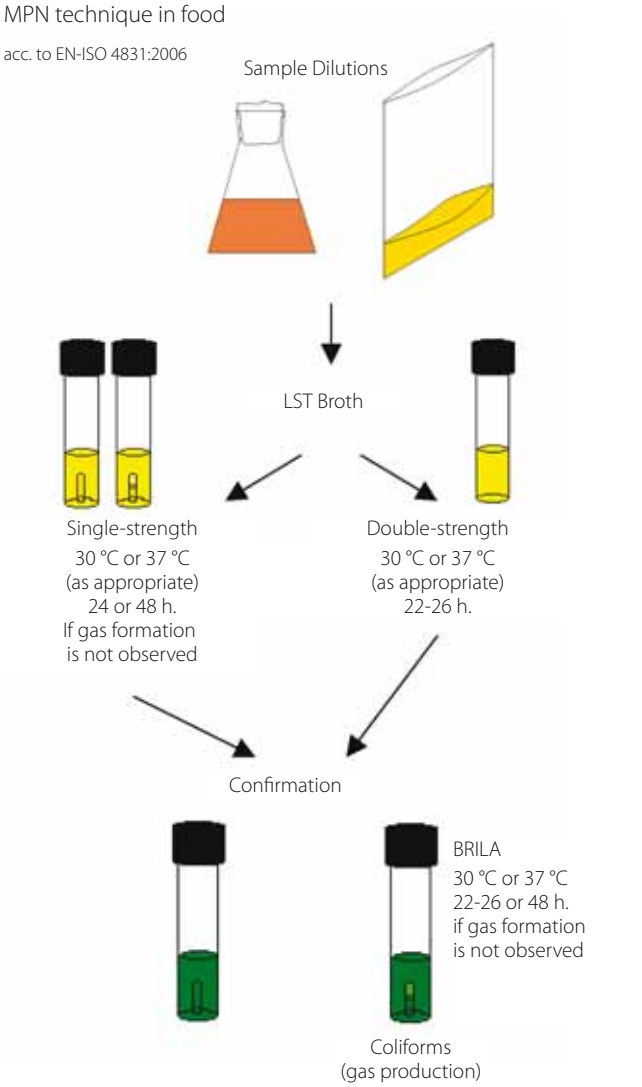
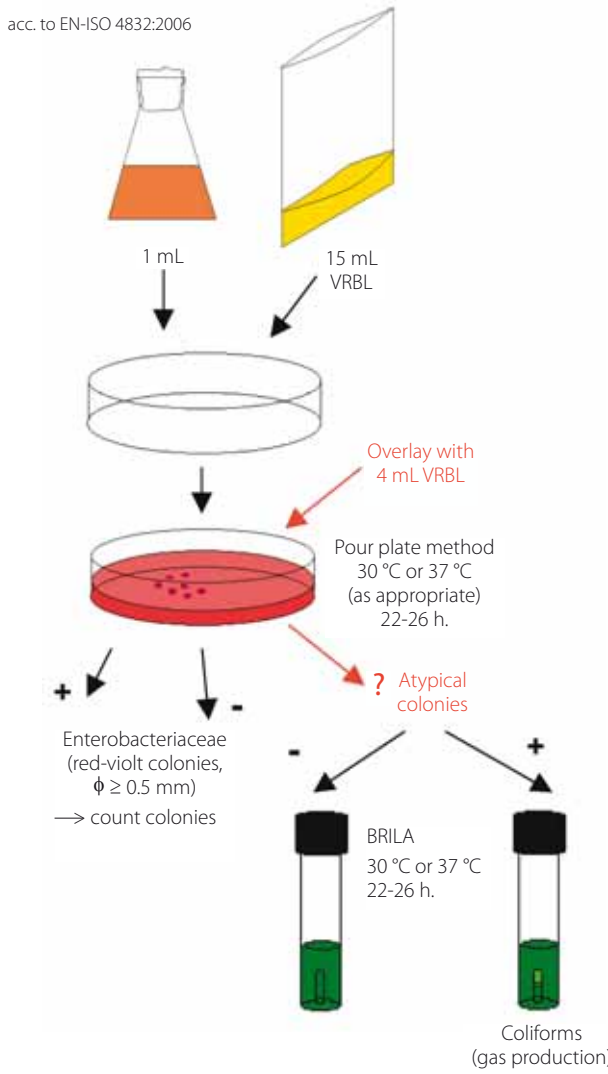


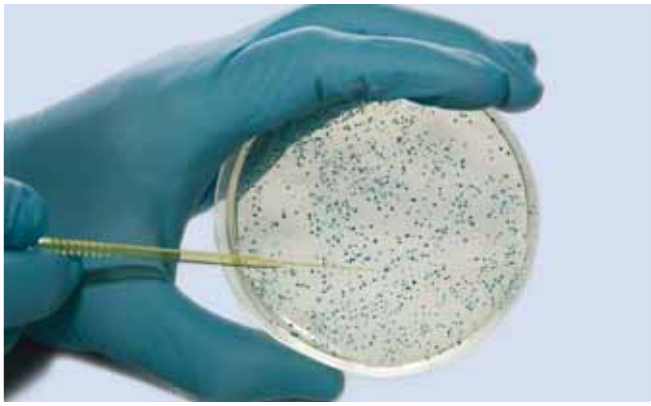
Figure 3: Method for the enumeration of coliforms - Colony-count technique in food



Description	Cat. No.
LST (Lauryl Sulfate Tryptose Broth)	17349-500G
BRILA (Brilliant Green Bile Lactose Broth)	16025-500G

Description	Cat. No.
VRBL (Violet Red Bile Lactose Agar)*	70188-500G
BRILA (Brilliant Green Bile Lactose Broth)	16025-500G

* not available in USA



Did you know…

Coliforms constitute just a small percentage of natural intestinal flora?

For example, only about 10% of a calf's intestinal bacteria are coliforms. The number depends on the age and nutrition of the animal. While there are about 200 different species of microorganisms, coliforms are quite characteristic of faecal contamination.

Figure 1: *E .coli* on chromogenic media

Table 1: Chromogenic media for detection of coliforms and particularly *E. Coli*

Cat. No.	Name (Engl)	Description (Engl)
16016	BRILA MUG Broth*	Brilliant Green Bile Lactose Broth with the addition of the fluorogenic substrate MUG. It directly confirms the presence of *E. coli* and differentiates it from other coliforms.
44657	ECD MUG Agar	The bile-salt mixture in this *E. coli* Direct Agar extensively inhibits the non-obligatory intestinal accompanying flora. The cleavage of the fluorogenic substrate MUG and a positive indole test demonstrate the presence of *E. coli*.
81938	HiCrome™ Coliform Agar*	A selective chromogenic medium recommended for simultaneous detection of *E. coli* and total coliforms in water and food samples. Sodium lauryl sulfate inhibits Gram-positive organisms. The chromogenic mixture contains two chromogenic substrates, Salmon-GAL and X-glucuronide. The enzyme β-D-galactosidase produced by coliforms cleaves Salmon-GAL, resulting in the salmon to red coloration of coliform colonies. The enzyme β-D-glucuronidase produced by *E. coli* cleaves X-glucuronide. *E. coli* forms dark blue to violet colored colonies due to cleavage of both Salmon-GAL and X-glucuronide. The addition of tryptophan improves the indole reaction, thereby increasing detection reliability in combination with the two chromogens. To confirm *E. coli*, add a drop of Kovac's reagent (Fluka No. 60983) on the dark blue to violet colony. Formation of cherry-red color indicates the positive reaction.
70722	HiCrome™ *E. coli* Agar B*	Detection and enumeration of *E. coli* in foods without further confirmation on membrane filter or by indole reagent. Most of the *E. coli* strains can be differentiated from other coliforms by the presence of enzyme glucuronidase which is highly specific for *E. coli*. *E. coli* cells absorb X-glucuronide and the intracellular glucuronidase splits the bond between the chromophore and the glucuronide. The released chromophore gives the blue coloration of the colonies.
73009	HiCrome™ ECC Agar*	HiCrome ECC Agar is a differential medium recommended for the presumptive identification of *E. coli* and other coliforms in food and environmental samples. The chromogenic mixture contains two chromogens as X-glucuronide and Salmon-GAL. X-glucuronide is cleaved by the enzyme β-glucuronidase produced by *E. coli*. Salmon-GAL is cleaved by the enzyme galactosidase produced by the majority of coliforms, including *E. coli*. Color of *E. coli* colonies: blue/purple
85927	HiCrome™ ECC Selective Agar*	HiCrome ECC Selective Agar is a selective medium recommended for the simultaneous detection of *E. coli* and coliforms in water and food samples. The ingredients help even the sublethally injured coliforms to grow rapidly, Tergitol inhibits Gram-positive as well as some Gram-negative bacteria other than coliforms. The chromogenic mixture contains two chromogenic substrates as Salmon-GAL and X-glucuronide. The enzyme β-D-galactosidase produced by coliforms cleaves Salmon-GAL, resulting in the salmon to red coloration of coliform colonies. The enzyme β-D-glucuronidase produced by *E. coli* cleaves X-glucuronide. *E. coli* forms dark blue to violet colored colonies due to cleavage of both Salmon-GAL and X-glucuronide. The addition of tryptophan improves the indole reaction. To confirm *E. coli*, add a drop of Kovac's reagent (Fluka No. 60983) on the dark blue to violet colony. Formation of cherry-red color indicates the positive reaction.
09142	HiCrome™ ECD Agar with MUG*	For the detection of *E. coli* in water and food samples by using a combination of chromogenic and fluorogenic substrate. The presence of *E. coli* is indicated by blue coloured colony formation due to cleavage of chromogenic substrate. The fluorogenic substrate MUG permits rapid detection of *E. coli* and also detects anaerogenic strains which may not be detected in conventional procedures.
90924	HiCrome™ m-TEC Agar*	HiCrome M-TEC Agar is recommended by the U.S. Environmental Protection Agency (USEPA) for differentiation and enumeration of thermotolerant *E. coli* from water by membrane filtration technique.
51489	HiCrome™ Rapid Coliform Broth*	Rapid HiColiform Broth is used for detection and confirmation of *E. coli* and coliforms on the basics of enzyme substrate reaction from water samples, using a combination of chromogenic and fluorogenic substrate. The fluorogenic substrate (MUG), is split by enzyme β-D-glucuronidase, which is specifically found in *E. coli*. The reaction is indicated by a blue fluorescence under UV light. The presence of total coliforms is indicated by a blue green color of the broth due to cleavage of chromogenic substrate (X-Gal). IPTG amplifies enzyme synthesis and increases the activity of β-D-galactosidase. To confirm presence of *E. coli*, by indole reaction, overlay the medium with Kovac's reagent (Fluka 67309). The layers turn red within 2 minutes in the case of positive reaction.
62634	LST-MUG Broth	LST broth with the addition of fluorogenic substrate MUG for the detection of *E. coli*. In addition, the medium is modified with tryptophan for a confirmation step with the Kovac's reagent (indole test).
39734	Membrane Lactose Glucuronide Agar	M-Lauryl Sulfate Chromogen Agar is for the differentiation and enumeration of *E. coli* and other coliforms, which simplifies the membrane filtration technique for *E. coli* and coliforms by reducing the number of filtration stages required from two to one, thereby reducing the need for further confirmation steps. Coliform bacteria are detected based on the fermentation of lactose fermentation (yellow colonies). X-Glucuronide is cleaved by β-glucuronidase present in the *E. coli*. In combination with the lactose fermentation, it results in a green colony.
M1678	MUG EC Broth	Selective media used for the detection of *E. coli* by a fluorogenic procedure.
17165	MUG Tryptone Soya Agar	For cultivation of fastidious and nonfastidious microorganisms, especially for *E. coli* by fluorogenic method.
51413	Plate Count MUG Agar	Plate Count MUG Agar is used for determination of plate count of microorganisms in milk and other dairy products, including direct detection and enumeration of *E. coli* by fluorogenic method.
92435	TBX Agar	Tryptone Bile Agar with the chromogenic substrate X-glucornide for the detection and enumeration of *E. coli* in foodstuffs, animal food and water without further confirmation.
95273	VRB MUG Agar	A Violet Red Bile Agar for the detection and enumeration of coliform bacteria, modified with the fluorogenic substrate to differentiate *E. coli*. Gram-positive accompanying flora is extensively inhibited by crystalviolet and bile salts. A colour-change to red indicates lactose-positive colonies, within which *E. coli* can be demonstrated by fluorescence in the UV.

* not available in USA

Carbohydrate and Yeast Extracts

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

Natural based ingredients are important ingredients for the media

Carbohydrate Extracts:

Natural based ingredients are complex ingredients that, in many cases, are important for the growth of fastidious microorganisms. Carbohydrate extracts are a significant nutritive complex component of media for the growth of organisms. While the carbohydrates serve primarily as an energy source with the carbohydrate but there are also protein and other compounds in these extracts. **Table 1** provides additional details regarding carbohydrate extracts.

Table 1: Carbohydrate Extracts

Cat. No.	Carbohydrate Extracts	Description
31405	Dextrin from potato starch	Dextrin is produced by hydrolysis of the starch. It is a good carbohydrate base for the growth of yeasts and moulds, as well as for lactobacilli and other bacteria.
70167	Malt extract	Extract of malted barley used especially as a basic ingredient for the cultivation of moulds and yeasts.
07915	Potato Extract	The major part of this extract is composed of carbohydrates. It is also rich in proteins, peptides and natural minerals. It is recommended to be used in Potato Glucose Agar for the detection and enumeration of yeasts and moulds. In addition, *Bordetella*, mycobacteria and other organisms like this nutritive source of potato.

Yeast Extracts:

Yeast extracts are produced under controlled conditions to retain their vitamin content and other nutritive values such as free amino acids. They are rich in vitamins, especially those belonging to the B complex group, and are often used to supply these factors in culture media at a concentration of 0.3-0.5 %. Yeast extracts are used particularly in media for cultivation of microorganisms. They are an important source of nitrogen (amino acids, peptides) but also serve as a source of carbohydrates. The quality of various yeast extract product differs primarily according to the nitrogen content, solubility, water and ash content. In addition, the different manufacturing processes and methods of lysing the cells have an influence on the growth of the organisms. **Table 2** presents an overview of the products available.

Table 2: Yeast Extracts

Cat. No.	Product	Specifications
73145	Yeast Autolysate	Total nitrogen ≥ 11% Amino-N ≥ 3.5% Loss on drying ≤ 5% Ash ≤ 15% Solubility (2% in water) yellow, clear
70161	Yeast Extract	Total Nitrogen ~11% Amino Nitrogen ~5% Loss on drying ≤ 6% Ash ≤ 15% Solubility (2% in water): yellow, clear
92144	Yeast Extract	Total nitrogen ≥ 10% Amino-N ≥ 4.5% Loss on drying ≤ 8% Ash ≤ 15% Solubility (2% in water) light yellow to brownish yellow, clear
09182	Yeast Extract, for technical purposes	Total nitrogen ≥ 9% Amino Nitrogen ≥ 3% Loss on drying ≤ 9% Ash ≤15% Solubility (2% in water): yellow, turbid non-dissolved <8%

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