Chromocult® Coliform Agar ES (Enhanced Selectivity)

Selective agar for the simultaneous detection and enumeration of total coliforms and *E. coli* in foods.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 041002

Intended Use

Chromocult® Coliform Agar ES is intended for use in Microbiology laboratories analyzing food and animal feeds. This chromogenic culture medium is selective and differential and enables *E. coli* and coliforms from food matrices, such as raw ground beef, raw ground chicken and raw milk, to be detected, differentiated and enumerated within 24 hours.

Fresh food usually contains a high microbial load and generally does not contain stressed or injured bacteria. The high level of accompanying flora requires higher selectivity of the culture medium to ensure inhibition of unwanted bacteria and allow the target organisms to grow well.

It was necessary to modify the well established Chromocult® Coliform Agar to fulfil this difficult and critical requirement. Exchanging Tergitol®7 with a combination of bile salts and propionate results in the extensive inhibition of accompanying bacteria. Chromocult® Coliform Agar ES is a medium allowing the simultaneous detection of coliforms/*E. coli* in samples with high bacterial bioburden.

Chromocult® Coliform Agar ES is therefore the ideal medium for the detection of coliforms/*E. coli* in fresh foods.

Intended User

Microbiological examinations are to be performed by trained staff only.

Validation Studies

Chromocult® Coliform Agar ES has been validated by the AOAC™ Research Institute under the Performance Tested MethodsSM program for the analysis of raw ground beef, raw ground chicken and raw milk.

The most probable number (MPN) method for coliform bacteria and *E. coli* (AOAC™ official method 966.24) was used for method comparison testing.

The Chromocult® Coliform Agar ES method was found to be equivalent to the AOAC™ official method.

Mode of Action

The combination of suitable peptones and the buffering using MOPS allow rapid growth of coliforms and an optimal transformation of the chromogenic substrates. The amount of bile salts and propionate largely inhibit growth of Gram-positive and Gram-negative accompanying flora.

The simultaneous detection of total coliforms and E. coli is achieved using the combination of two chromogenic substrates. The substrate Salmon™-β-D-GAL is cleaved by β-D-galactosidase, characteristic for coliforms, resulting in a salmon to red colouration of coliform bacteria colonies. The substrate X-β-D-glucuronide is cleaved by β-D-glucuronidase, characteristic for *E. coli*, causing positive colonies to turn blue in colour.

As E. coli cleaves Salmon™-β-D-GAL as well as X-β-D-glucuronide, its colonies turn to a dark violet colour and are easily differentiated from the other coliforms having a salmon-red colour.

Typical Composition (g/litre)

Peptone 5.0; potassium chloride 7.5; MOPS 10.0; bile salts 1.15; propionate 0.5; agar-agar 10.0; 6-chloro-3-indoxyl-beta-D-galactopyranoside 0.15; isopropyl-beta-D-thiogalactopyranoside 0.1; 5-bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid 0.1.

Preparation

Suspend 34.5 g in 1000 ml of purified water and heat to boiling with frequent agitation until completely dissolved (approximately 45 minutes).

- Do not autoclave, do not overheat.

Immediately cool the medium in a water bath at 45-50 °C (a precipitate appears if a period of 2 hours is exceeded).

pH: 7.0 ± 0.2 bei 25 °C

The medium is clear and colourless.

Prepared plates can be stored in sealed plastic pouches or bags for up to 2 weeks at 2 - 8°C and protected from light.

Sample Preparation

Prepare test samples using standard laboratory techniques such as those described in the Bacteriological Analytical Manual or appropriate ISO standard specific for the product concerned.

To minimize possible interference between the coloration of coliforms/E. coli and the sample (e.g. low pH), it is advisable to dilute the sample 1:10 in a buffered solution (e.g. add 450 ml Butterfield's phosphate buffer, buffered peptone water, or buffered sodium chloride peptone broth to blender jar containing 50 g of sample and mix for 2 min); perform further dilutions as needed.

Application

Chromocult® Coliform Agar ES is usually inoculated by the pour plate method. Using a sterile pipette, transfer 1 ml of the liquid test sample (or 1 ml from the appropriate dilution) into a sterile Petri dish.

Pour approximately 15 ml of Chromocult® Coliform Agar ES into each Petri dish while the medium is still liquid, but only after the medium is cooled to 45-50°C. Carefully swirl the plate until the inoculum is thoroughly mixed with the medium. Allow the mixture to solidify on a cool horizontal surface.

Incubate the inoculated dishes aerobically at 35-37°C in an inverted position (agar side up) for 24 hours. After incubation, examine the plates for the presence of typical colonies of *E. coli* and other coliforms.

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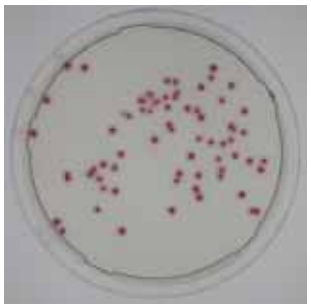
Results

Count the dark blue to violet colonies as *E. coli* and the salmon to red colonies as other coliforms.

The total of all red and blue colonies represent the total coliform count.

Some *E. coli* (3-4%) are β-glucuronidase-negative and grow as salmon-red colonies, e.g. *E. coli* O157 strains.



E. coli 0157 ATCC 1844

Accompanying flora appears as colourless colonies, except for some organisms, which possess β-D-glucuronidase activity. These colonies appear light blue to turquoise in colour.

Salmonella Urbana ATCC 9261

The total coliform count should not exceed 150 typical CFU and 300 total CFU (total coliforms and accompanying bacteria) per plate. Above these levels, the colonies cannot be counted accurately. Samples, which are expected to exceed these maximum levels, should be diluted prior to inoculation.

Calculate the number of E. coli and other coliforms per milliliter or per gram of sample from the number of characteristic colonies in the plates.

Limitations

Plates have to be dry before use. In case the agar surface is wet, plates inoculated with food samples using the surface spreading method might produce poorly distinguished, uncharacteristic colonies that are difficult or impossible to enumerate.

For food samples use the pour plate technique as the preferred method.

Performance Characteristics

Chromocult® Coliform Agar ES was evaluated for the recovery of coliforms/E. coli in raw ground beef, raw ground chicken and raw milk in internal and AOAC™ approved external laboratories.

The study compared the Chromocult® Coliform Agar ES method to the AOAC™ reference method (966.24) for the enumeration of E. coli and coliform bacteria. The food samples were pre-tested to establish the titre of naturally contaminating E. coli and coliform bacteria. Three 300 g samples of raw ground beef, and raw ground chicken were batch-inoculated with dry inoculum, each at a different level: "low" (targeted spike of 10 CFU/g), "middle" (targeted spike of 10^2 CFU/g), and "high" (targeted spike of 10^3 CFU/g). One additional 300 g sample remained uninoculated to serve as a negative control.

Raw milk was not inoculated. Initial coliform counts for raw milk represent the "low" level for both E. coli and coliform bacteria. The milk was then subjected to temperature treatment for 3 hours at 35°C to obtain a "middle" bacteria level and an additional 3 hours at 35°C to obtain a "high" bacteria level.

From each 300 g sample, five 50 g sub samples were weighed and blended with 450 mL of sterile diluent (Butterfield's phosphate buffer) for 2 minutes.

Following blending, five 50 g samples were plated individually onto Chromocult® Coliform Agar ES. Dilutions were made using 99 mL of Butterfield's phosphate buffer as diluent. Both, the pour plate and spread plate technique was used for all foods. Plates were inverted and incubated aerobically at 35°C for 24 ±1 hours. Enumeration of dark-blue or violet colonies yielded a presumptive E. coli count while enumeration of salmon or red-coloured colonies yielded a presumptive coliform count. Confirmation of typical E. coli colonies was done according to AOAC™ method 966.24, followed by Gram stain, oxidase test, and analysis with MicroID test strips.

Each 50 g sub sample plated onto Chromocult® Coliform Agar ES was also tested using a 3-tube, most probable number (MPN) series. The AOAC™ official method 966.24 "Most Probable Number Method for Coliform Bacteria and E. coli" was followed. Typical E. coli colonies on L-EMB were confirmed by Gram stain, oxidase test, and analysis with MicroID test strips.

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Merck's Chromocult® Coliform Agar ES effectively detected total coliforms and E. coli in all food types tested. Independent of the spike level, the coliform and E. coli level determined by AOAC™ MPN method 966.24 was consistent with results generated by the Chromocult® Coliform Agar ES for all foods. Furthermore, presumptive E. coli colonies on Chromocult® Coliform Agar ES (violet in colour) were confirmed as E. coli in all isolates. Similarly red, presumptive coliform colonies all produced gas in BGLB, indicative of confirmed coliform identification. This suggests that Chromocult® Coliform Agar ES not only can reliably quantify total coliforms present in a food, but also consistently differentiate E. coli from other coliform bacteria.

Fifty three (53) pure cultures of E. coli and other coliforms were cultured on Chromocult® Coliform Agar ES at 35°C for 24 hours. All strains of E. coli and other coliforms showed characteristic growth. Overall sensitivity for Chromocult® Coliform Agar ES is 100%.

Forty four (44) pure cultures of non-coliform bacteria were cultured on Chromocult® Coliform Agar ES at 35°C for 24 hours. Most of the non-coliform bacteria showed colourless growth or were completely inhibited. Only 3 strains showed turquoise coloration. There were no false positive reactions. Overall specificity for Chromocult® Coliform Agar ES is 100%.

Storage conditions and Shelf life

Store the dehydrated medium dry and tightly closed. Protect from light. Don't use clumped or discoloured medium. Store at +15°C to +25°C and use before expiry date shown on the label.

Precautions

Both, contaminated and not used culture media must be disposed in a way which is safe and meets state or national regulations. The Material Safety Data Sheet (MSDS) provides detailed information on disposal of each medium. Safety information on ingredients and culture media are summarised in Merck's ChemDAT manual. The ChemDAT manual is available on CD-ROM or can be down loaded from Internet www.chemdat.info.

Technical assistance

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Website: www.merck.de

Literature

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - General rules for microbiological examinations. ISO 7218:1996/Amendment 1:2001.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions. ISO 6887-1:1999.

Kilian, M. and Bülow, P. 1976. Rapid diagnosis of Enterobacteriaceae. I. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sect. B 84: 245-251.

U.S. Food and Drug Administration. 2003. Bacteriological Analytical Manual Online, Chapter 1: Food Sampling and Preparation of Sample Homogenate, http://www.cfsan.fda.gov/~ebam/bam-1.html, accessed June 24, 2008

Ordering Information

Product	Ordering No.	Pack size
Chromocult® Coliform Agar ES (Enhanced Selectivity)	1.00850.0500	500 g
Buffered Peptone Water (BPW)	1.07228.0500	500 g
Sodium chloride peptone broth (buffered)	1.10582.0500	500 g

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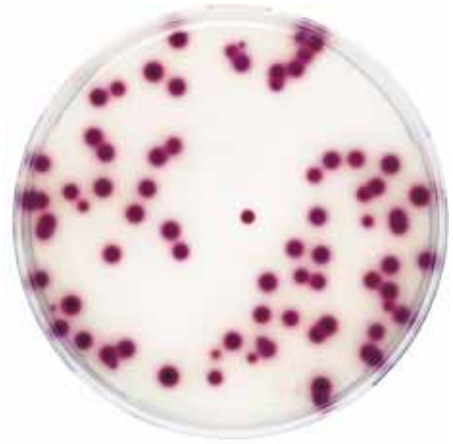
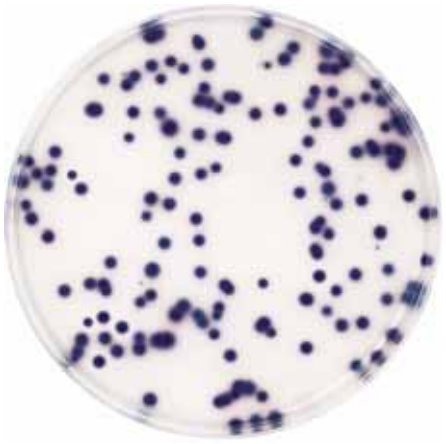
Quality control

Test strains	Inoculum (CFU/plate)	% Recovery	Colony colour
E. coli ATCC 11775	10-100	≥ 70	dark blue to violet
Citrobacter freundii ATCC 8090	10-100	≥ 70	salmon-red
Enterobacter cloacae ATCC 13047	10-100	≥ 70	salmon-red
Salmonella typhimurium ATCC 14028	10-100	not limited	colorless
Serratia liquefaciens ATCC 27592	$>10^4$	≤ 0.01	
Staphylococcus aureus ATCC 25923	$>10^4$	≤ 0.01	
Lactococcus lactis ATCC 19435	$>10^4$	≤ 0.01	
Bacillus subtilis ATCC 6633	$>10^4$	≤ 0.01	



Citrobacter freundii ATCC 8090



E. coli ATCC 11775