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Instructions for Use

CRITERION™ HARDYCHROM™ ECC COMPLETE

Cat. no. C9030	CRITERION™ HardyCHROM™ ECC Complete	52.8g
Cat. no. C9031	CRITERION™ HardyCHROM™ ECC Complete	500g
Cat. no. C9032	CRITERION™ HardyCHROM™ ECC Complete	2kg
Cat. no. C9033	CRITERION™ HardyCHROM™ ECC Complete	10kg
Cat. no. C9034	CRITERION™ HardyCHROM™ ECC Complete	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ HardyCHROM™ ECC Complete is recommended for the detection, differentiation, and enumeration of *Escherichia coli* and coliforms in food or water samples based on colony color.

Dehydrated culture media is a raw material not intended for use in the diagnosis of human disease. For implementation, this product requires additional processing and supplementation of ingredients before use.

SUMMARY

Routine testing to assess the sanitary quality of food or water is performed by detecting and enumerating indicator organisms rather than pathogens. The coliform group of organisms are recognized as the principal indicator of unsanitary conditions. Coliforms are characterized as gram-negative, lactose-fermenting rods. They are present in the intestinal tract of mammals, including man, and other animals. Non-fecal coliforms are found in many areas of the environment, including in soil and on plants. CRITERION™ HardyCHROM™ ECC Complete allows for the detection of *E. coli* and other coliforms when testing.

E. coli can be identified as pink to violet colored colonies on the medium, while other coliform bacteria will appear as turquoise colonies. Organisms other than coliforms or *E. coli* (including approximately 4% of *E. coli* strains and most O157 strains) will appear as white or colorless colonies. Further testing is recommended for complete identification.

Hardy Diagnostics CRITERION™ HardyCHROM™ ECC Complete contains peptones to provide essential growth substances and trace ingredients. Sodium chloride maintains osmotic equilibrium. CRITERION™ HardyCHROM™ ECC Complete also contains chromogenic substrates which allow specific microorganisms to be recognized by their colony color. Selective agents have been added to inhibit the growth of gram-positive and other non-coliform bacteria present in the sample.

FORMULA*

Gram weight per liter:	26.4g/L
Peptones	9.3g

Selective Agents	4.52g
Sodium Chloride	2.0g
Chromogenic Mixture	1.0g
Agar	10.0g

Final pH 7.0 +/- 0.3 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Store the sealed bottle(s) that contain dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic and will clump when exposed to moisture and air. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. Dehydrated culture media should be discarded if clumped, if the media is not free-flowing or if the color has changed from its original beige. Do not remove the container desiccant, if applicable.

Store the prepared culture media at 2-8ºC and protect from light or heat prior to use.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 26.4g of the dehydrated culture media in one liter of distilled or deionized water. Stir to mix thoroughly.

2. Boil for one minute to dissolve completely.

3. DO NOT AUTOCLAVE

4. Dispense medium into pre-sterilized petri dishes.

Note: The shelf life of in-house prepared media from dehydrated culture media is dependent upon preparation methods,

container quality, equipment, storage conditions, and batch testing criteria and must be validated by the end user. Refer to *USP Microbiological Best Laboratory Practices <1117>* for more information on validation procedures.[1]

PROCEDURE

Specimen Collection: Consult listed references for information on sample collection.[1-6] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the sample should be inoculated to an appropriate transport medium and refrigerated until inoculation.

Method of Use: Allow plates to warm to room temperature. The agar surface should be dry prior to inoculating. Inoculate and streak the sample as soon as possible after collection.

1. Direct Inoculation -

a. If the sample is on a swab, roll the swab over a small area of the agar surface.

b. Streak for isolation with a sterile loop.

c. Follow step 5 below.

2. Spread Plate Method -

a. Prepare dilutions in sterile diluent to obtain 30-300 CFU per plate.

b. Aseptically inoculate agar surface with 0.1ml of well mixed diluted sample.

c. Using a sterile spreader device, distribute the inoculum evenly over the agar surface.

d. Follow step 5 below.

3. Membrane Filtration Method -

a. Prepare dilutions in sterile diluent to obtain 20-80 CFU.

b. Pipet 1ml of dilution to a vial of Butterfield's Buffer (Cat. no. D599) or Phosphate Buffer with MgCl2 (Cat. no. D699).

c. Shake well.

d. Using membrane filter apparatus, filter buffer through the membrane filter.

e. Aseptically remove the filter and place on the agar surface.

f. Follow step 5 below.

4. Contact Plates -

a. Hold the plate with thumb and second finger and use index finger to press plate bottom firmly against the selected test surface.

b. The same amount of pressure should be applied for every sample. Do not twist or move the plate laterally. Lateral movement spreads contaminants over the agar surface, thus making resolution of colonies difficult.

c. A rolling motion may be used for slightly curved surfaces.[1-5]

d. Section or grid areas (walls, floors, etc.) to be assayed. Samples can then be taken from specific points within the grid.

e. Follow step 5 below.

f. Using adequate light and magnification, count the number of colonies within the squares of the grid area. Take care not to count a square more than once. Using a Bactronic or Quebec colony counter, count colonies and record as the number of colonies per contact plate or number of colonies per square centimeter.[1-5]

g. Data should be collected and recorded according to a designed monitoring system that statistically provides for the accurate acquisition of data for multiple samples over time.

5. Incubate plates aerobically at 35-37°C for 18 to 24 hours. Observe plates for characteristic colonial morphology and color (some organisms may take longer than 24 hours for visible growth to appear).

Note: If research is focused on fecal coliform bacteria, incubate plates at 44°C.

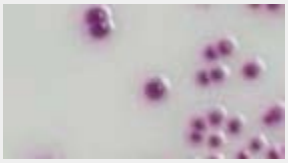
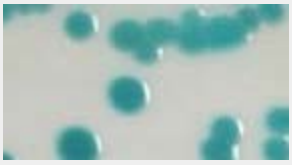
INTERPRETATION OF RESULTS

Following incubation, examine the plates for growth. Count the number of colonies and express in number of colony forming units (CFU) per gram or milliliter of sample. Take into account the dilution factor. If duplicate plates were set-up, express the average for the two plates in terms of the number of microorganisms per gram or milliliter of sample. Consult references for additional information on interpretation and enumeration of microbial growth on this medium.

Pink to violet colored colonies are a positive test for the presence of *E. coli*.

Turquoise colonies are a positive test for the presence of coliform bacteria other than *E. coli*.

Other gram-negative bacteria appear as white or colorless colonies. Gram-positive bacteria are inhibited.

Organism	Description	Photo	Color
Escherichia coli	pink to violet colonies		
Klebsiella spp., *Enterobacter* spp.	turquoise colonies		

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Organisms other than coliforms or *E. coli* (including approximately 4% of *E. coli* strains and most O157 strains) will appear as white or colorless colonies.

Color-blind individuals may encounter difficulty in distinguishing the color differences on CRITERION™ HardyCHROM™ ECC Complete.

Accurate counting may be difficult with molds or spreading colonies.

Rare, fastidious microorganisms may not grow on selective media formulations.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, incubators, tubes, bottles, petri dishes, media such as dilution vials such as Butterfield's Buffer (Cat. no. D599) or Phosphate Buffer with MgCl2 (Cat. no. D699), etc., are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	A	24hr	35°C	Aerobic	Growth; pink to violet colonies
Klebsiella pneumoniae ATCC® 13883	A	24hr	35°C	Aerobic	Growth; turquoise colonies
Enterobacter cloacae ATCC® 23355	A	24hr	35°C	Aerobic	Growth; turquoise colonies
Enterococcus faecalis ATCC® 29212	B	24hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

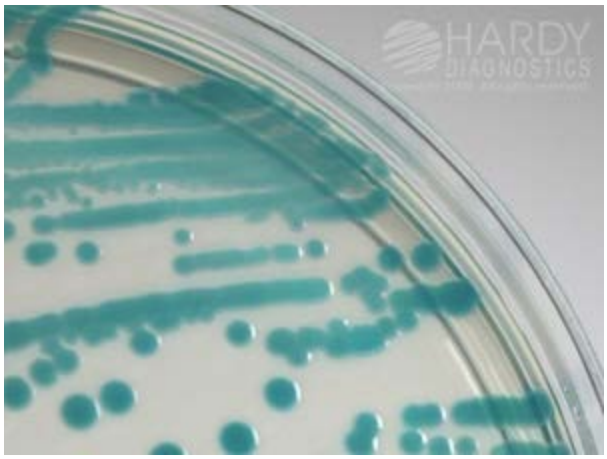
USER QUALITY CONTROL

Users of dehydrated culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ HardyCHROM™ ECC Complete powder should appear homogeneous, free-flowing, and beige in color. The prepared medium should appear opalescent, and light amber in color

Escherichia coli (ATCC® 25922) colonies growing on CRITERION™ HardyCHROM™ ECC Complete incubated aerobically for 24hrs at 35°C.



Klebsiella pneumoniae (ATCC® 13883) colonies growing on CRITERION™ HardyCHROM™ ECC Complete incubated aerobically for 24hrs at 35°C.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater.* APHA, Washington, D.C.

2. Association of Official Analytical Chemists. *Official Methods of Analysis*. AOAC, Washington, D.C.

3. American Public Health Association. *Standard Methods for the Examination of Dairy Products.* APHA, Washington, D.C.

4. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods.* APHA, Washington, D.C.

5. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

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Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality

management system is certified to ISO 13485.