

OPERATOR'S MANUAL



PeelPlate® CC-C HV
COLIFORM FOR HARD CHEESE HIGH VOLUME

FOR DETECTION AND ENUMERATION OF TOTAL COLIFORM BACTERIA

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Kit Information

INTRODUCTION

The Peel Plate® Coliform Count for Hard Cheese High Volume (CC-C HV) test detects and enumerates coliform bacteria in 5 mL sample volumes of pH adjusted cheese homogenates. The method is applicable for determination of total coliform, in certain hard cheeses such as Swiss, Parmesan, Romano, Asiago, Muenster, and Provolone, when incubated at 32 ± 1 °C for 24 to 48 hours. A 5 mL aliquot of a 1:10 sample dilution (11 gram cheese to 99 mL buffer) is pH adjusted to 7.5 and added to the CC-C HV plate and is incubated at 32 ± 1 °C. The Peel Plate CC-C HV test is intended for microbiological laboratories, but may also be used by food quality stakeholders such as farmers, and cheese processors. The method limit of detection is 1 or greater coliform colony forming units per 5 milliliter (CFU/5 mL or 2 CFU/1.0 g cheese) of test sample. The quantitative range for coliform is defined as 1 to 154 CFU/plate as defined in FDA-BAM manual.

KIT CONTENTS, STORAGE, AND TESTING CONDITIONS

A 100 test kit (Kit Code: PP-CC-C-HV-100K) contains 4 foil bags, each with 25 Peel Plate CC-C HV formulation tests.

Store kits in foil bags for up to 12 months in refrigerator or at room temperature for up to 1 month.

Open bag and perform testing in a clean dry testing area at ambient temperature. Remove number of plates need for analysis. Reseal the bag using the zip closure to store unused tests. Moisture or heat or storage abused test will discolor yellow. Do not use discolored tests or tests from bags with pink/white desiccant indicator.

PRINCIPLE

The Peel Plate CC-C HV medium is based on conventional coliform selective medium to support and colorimetrically differentiate coliform in test samples. The Peel Plate CC-C HV test contains the enzyme substrates salmon-gal (6-chloro-3-indolyl-B-D-galactopyranoside) used to detect β -galactosidase enzyme produced by coliform as well as some performance optimization additives to reduce background red color caused by culture bacteria and cheese enzymes. It also contains gelling and wicking agents which absorb and self-diffuse the sample.

APPLICABILITY

The Peel Plate CC-C HV is applicable specifically to certain cheeses and their shredded blends noted in the introduction or any cheeses that produce a strong red background on the standard Peel Plate CC HV and EC HV tests.

PRECAUTIONS:

- Observe Good Laboratory Practices for microbial testing. Avoid specimen contamination.
- Perform tests with clean washed and gloved hands assuming potential pathogenic bacteria.
- Test on a level surface in a clean area, free of dust and draft-free.
- Avoid hand contact with test samples and Peel Plate CC-C HV medium.

Sample Preparation

CHEESE

- Add 11 g of cheese (Swiss, Parmesan, Romano, Asiago, Muenster, Provolone and their blends) to 99 mL of microbiologically suitable dilution blanks.
- Homogenize or stomach for 2 minute.
- Let settle and decant supernatant to a container for pH adjustment.
- pH adjust using pH paper or based on product experience. Add 1N NaOH in 0.1 mL increments to achieve a pH~7.5 of the homogenate. With hard cheeses it takes approximately 0.5 to 1.0 mL to pH adjust 80 mL of decanted homogenate.

Procedure

**Step 1**

- Apply pressure to back side of plate and pull up the cover tab.
- Lift cover to expose the Peel Plate CC-C HV culture disc.

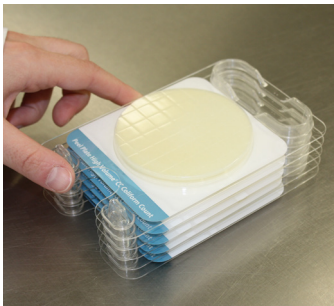
**Step 2**

- Pipet vertically and rapidly 5.0 mL of homogenate onto the center of exposed plate containing the Peel Plate CC-C HV medium. Expel pipet contents rapidly with even force and within 2 to 3 seconds.



Step 3

- Sample should diffuse to edges of the plate.
- Re-apply the cover and avoid wrinkling.
- Allow plate to sit 30 seconds before moving.



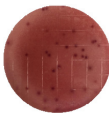
Step 4

- Incubate plates with lid down as shown.
- Incubate at 32 ± 1 °C for 24 hours.
- Observe plates for growth and red background. With strong red background incubate an additional 24 hours.
- Plates stacked 12 high do not affect plate heat transfer.

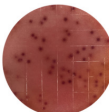
Analysis of Results

Examples of coliform spiked cheeses incubated for 24 hours.

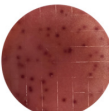
Munster



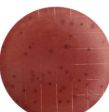
Provolone



Parmesan



5 Blend



- At the end of incubation period, observe plates for colonies viewed through the bottom side of the plate.
- Some cheeses may present a stronger reddish background at 24 hours and will require an additional 24 hour incubation for coliform quantitation, e.g. Swiss, Romano, and some Parmesan
- Each red spot represents one CFU. The sum of spots is reported as the total coliform CFU/5 mL of the diluted sample (2 CFU/1.0g cheese).
- Sum the results of two 5 mL plates of 1:10 dilution to calculate coliform CFU/gram of cheese.
- Counts of 1 to 154 CFU/5 mL are considered in countable range, while counts outside that range are considered estimates. Samples with results outside of countable range (>154 CFU/mL) can be diluted and retested.
- Some cheese present a stronger reddish background at 24 hours and require an additional 24 hour incubation for coliform quantitation, e.g. Swiss, Romano and some Parmesan.

Interpretation of Results

- The Peel Plate CC-C formulation is very similar to Peel Plate EC formulation except the *E. coli* color indicator is removed. In Peel Plate EC test inclusivity and exclusivity studies, 57 of 58 coliform inclusivity isolates were correctly detected, including all 17 *E. coli* strains. The coliform strain not detected was *Escherichia blattae*-ATCC 29907. Of the 32 exclusivity strains evaluated, 31 were correctly excluded. The strain that was detected as coliform was, *Shigella sonnei*, ATCC 9290.
- Peel Plate CC-C HV tests have been evaluated in cheeses mentioned in this protocol, but have not been evaluated with all cheeses. Other types of cheeses may present reddish background requiring 48 hour incubation.

Quality Control

Quality control should be performed according to Good Laboratory Practices, and with the frequency determined by laboratory standard operating procedures. Common practices call for a Dilution Control, Negative Control, and Positive Control.

- **Dilution Control:** Test 5.0 mL of sterile dilution buffer to verify no detectable bacteria after incubation.
- **Negative Control:** Prepare Negative Control by autoclaving the appropriate dilution of test sample at 121 °C for 15 minutes. Cool to 4 °C and test 5.0 mL. Verify no detectable coliform bacteria in the Negative Control.
- **Positive Control:** To prepare, spike a sample with known coliform culture. Dilute sample to countable range of 1 to 154 CFU/5 mL and test 5.0 mL to verify detection after incubation.

Disposal

Microbiological cultures and reagents should be collected in biohazard bags and autoclaved. Dispose according to local, state, and federal regulations.

Technical Support

For questions contact a local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.

Order Information

Peel Plate CC-C HV tests are supplied in 25 (Kit code: PP-CC-C-HV-25K) and 100 (Kit code: PP-CC-C-HV-100K) test kits.

The Peel Plate test is also available in 1 mL sample volume tests in 100 and 1000 test kits for Aerobic Count, *E.coli*/coliform, Enterobacteriaceae, Yeast/Mold and Heterotrophic Plate Count. Refer to the Charm web site www.charm.com for more information.

Warranty

Charm Sciences, Inc. ("Charm") warrants each reagent product, including but not limited to test kits, to be free from defects in materials and workmanship and to be free from deviations from the specifications and descriptions of Charm's reagent products appearing in Charm's product literature, when stored under appropriate conditions and given normal, proper and intended usage, until the expiration of such reagent product's stated shelf life, or, if none is stated, for one year from the date of delivery of such reagent product to the end-user purchaser. THIS WARRANTY IS IN LIEU OF ALL OTHER WARRANTIES, WHETHER STATUTORY, EXPRESS, IMPLIED (INCLUDING WARRANTIES OF TITLE, NON-INFRINGEMENT, MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE AND ALL WARRANTIES ARISING FROM COURSE OF DEALING OR USAGE OF TRADE). The warranty provided herein may not be altered except by express written agreement signed by an officer of Charm. Representations, oral or written, which are inconsistent with this warranty are not authorized and if given, should not be relied upon. In the event of a breach of the foregoing warranty, Charm's sole obligation shall be to replace any reagent product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Charm promptly of any such defect prior to the expiration of said warranty period. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Charm is willing to replace any nonconforming reagent product or part. Charm shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damages sustained by any customer from the use of its reagent products. Except for Charm's obligation set forth above to replace any reagent product that proves defective within the warranty period, Charm shall not be liable for any damages of any kind arising out of or caused by any incorrect or erroneous test results obtained while using any such reagent product, whether or not caused by a defect in such reagent product.

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